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PERNICIOUS ANEMIA.

THE ERYTHROCYTE RESPONSE TO TREATMENT.

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SOON after their discovery of the therapeutic effectiveness of liver in pernicious anemia, Minot and his associates[3,4] recognized certain quantitative relationships between the erythrocyte count before treatment, the dose of liver employed in treatment, the number of reticulocytes which appeared early in remission, and the increase in the erythrocyte count. They were impressed particularly by the importance of the large number of reticulocytes in the blood in the first phase of the remission following liver therapy. They and others[1,3,4,6] devised mathematical equations which represent the numbers or percentage of reticulocytes which may be expected to appear in individual patients with pernicious anemia who are adequately treated. Although these standard reticulocyte equations have proven useful in the estimation of the clinical effectiveness of the various therapeutic agents, in the regulation of their dosage, in the clinical management of patients and in the assaying of materials of unknown therapeutic potency, their value for these purposes has been disputed, particularly by Murphy.[5a,b] He questions the accuracy of the reticulocyte response as a quantitative index of the amount or potency of therapeutic agents used in pernicious anemia. He believes the increased numbers of reticulocytes after treatment serve qualitatively to indicate the presence of active material in the substance used in treatment but cannot be used as a quantitative measure of the amount of the active material administered. This opinion is based upon the apparent lack of correlation in his cases between the amount of active liver

principle given and the magnitude of the reticulocyte response on one hand, and the magnitude of the erythrocyte increase on the other. He maintains that the increase in the numbers of erythrocytes during treatment is more accurate an index of the potency and amount of the active liver principle administered. Using the average increase in the erythrocyte count per day as an index of the effectiveness of treatment he finds, in his cases which received liver extract derived from 100 to 300 gm. of liver given intramuscularly during the first week of treatment, that the average daily increase in the erythrocyte count during the first 2 weeks of treatment varies from 128,000 to 54,000 per c.mm. of blood in patients whose initial erythrocyte counts ranged from 1 to 3.5 millions per c.mm. The highest values were found when the erythrocyte count was low.

Obviously, the rise in the erythrocyte count in response to therapy would be a far simpler method of evaluating the effect of treatment than the reticulocyte response if the expected erythrocyte increase were expressed in as simple and accurate form as the standard reticulocyte equations. With this in mind a large number of remissions have been studied to determine the quantitative relationships between the rate of erythrocyte increase and the erythrocyte count before treatment.

Materials. The present study is devoted to an analysis of the quantitative relationships existing in 600 patients with pernicious anemia, between the erythrocyte count before treatment and the increase in the numbers of erythrocytes after treatment. These patients had the typical clinical manifestations of pernicious anemia and the characteristic blood findings associated with this disease. A certain number, as would be expected in so large a group, had the various complications commonly associated with pernicious anemia, in varying degrees of severity. Rarely the presence of cancer of the stomach, surgical removal of large parts of the stomach, lesions of the liver and other conditions provided a possible anatomic cause for the hematologic and clinical picture of pernicious anemia. These patients had in common a macrocytic anemia which responded with the familiar reticulocyte response and an increase in the number of circulating erythrocytes when adequate amounts of therapeutic materials were given. To study the problem in all its manifestations every remission was recorded, whether satisfactory or not. All cases showing an increase in the number of reticulocytes above 3% were recorded. Among the 600 cases studied, 597 remissions were tabulated in which sufficient data were present to be of value. Various types of treatment were used. For convenience in analysis, therapy was listed under five classifications: 1, Intravenous liver extract; 2, intramuscular liver extract; 3, ventriculin; 4, oral liver extract; 5, miscellaneous.

In the last of these classifications the patients were treated with experimental extracts of liver, stomach and other organs given orally, rectally and parenterally; yeast products; gastric juice; and gastric juice and meat. Spontaneous remissions were also included in this group.

The data, red blood cell counts, reticulocyte estimations, cell measurements, and so on, were most abundant. These estimations were carefully performed by accepted methods, with standardized instruments, by competent technicians.

The Relationship Between the Increase in the Erythrocyte Count and the Initial Level of the Erythrocyte Count. Classifying the 597 remissions according to the type of treatment used, five groups were obtained. There were 114 patients who received liver extract orally, 149 who were given ventriculin, 85 who received liver extract intramuscularly and 175 to whom liver extract was administered intravenously. The remaining 74 patients had spontaneous remissions or were treated experimentally with various substances. This last group furnished variable data unsatisfactory for analysis. The remaining four groups were sufficiently large and homogenous to determine with considerable accuracy the relationships existing between the increase in the erythrocyte count and the initial erythrocyte count taken before treatment. To determine this relationship each of these groups was subdivided according to the erythrocyte count before treatment at 0.5 million intervals. Next each of these subdivisions was divided arbitrarily into three equal groups according to the percentage of reticulocytes present at the peak of the response. The patients in the subdivisions with the lowest reticulocyte percentages were arbitrarily considered to be "unsatisfactorily treated." The average weekly increase in the erythrocyte count for the remaining 349 patients "satisfactorily treated" was computed after the first 2 weeks of treatment. When the average weekly increase in the erythrocyte count in this group was plotted graphically (Fig. 1) the equation $I = 0.78 - 0.174Eo$* was obtained. In the same manner, the average weekly increase in the erythrocyte count for the "unsatisfactorily treated" group of 174 patients, the equation $I = 0.6 - 0.176Eo$ was obtained.

The results of this procedure when applied to the various treatment groups of "satisfactorily treated" cases, receiving oral liver extract, ventriculin, intramuscular and intravenous liver extract are shown in Table 1.

TABLE 1.—EQUATIONS REPRESENTING THE OBSERVED AVERAGE WEEKLY INCREASE IN THE ERYTHROCYTE COUNT IN VARIOUS GROUPS OF PATIENTS UNDER DIFFERENT TYPES OF TREATMENT AFTER 2 WEEKS OF TREATMENT.

Method of treatment.	
1. Oral liver extract, "satisfactorily treated"	$I = 0.78 - 0.177Eo$
2. Ventriculin, "satisfactorily treated"	$I = 0.70 - 0.171Eo$
3. Intramuscular liver extract, "satisfactorily treated"	$I = 0.78 - 0.175Eo$
4. Intravenous liver extract, "satisfactorily treated"	$I = 0.88 - 0.175Eo$
5. Average of all "satisfactorily treated cases"	$I = 0.78 - 0.174Eo$
6. Average of all "unsatisfactorily treated cases"	$I = 0.60 - 0.176Eo$

In these various equations, the positive values vary widely from 0.6 in the case of "unsatisfactorily treated" patients to 0.88 in the group of patients treated satisfactorily with intravenous liver extract. The numerical value of this positive factor appears to be

* I represents the average weekly increase in the erythrocyte count during the first 2 weeks of treatment, Eo the erythrocyte count before treatment in millions per c.mm. of blood.

directly proportional to the effectiveness of treatment. On the other hand, the negative values in the various equations are all approximately the same, the range of variation being from 0.171Eo to 0.177Eo and the average value being 0.174Eo. These negative values being uniform regardless of the type or effectiveness of treatment must have no relationship to the effectiveness of treatment and, being proportional to the erythrocyte count before

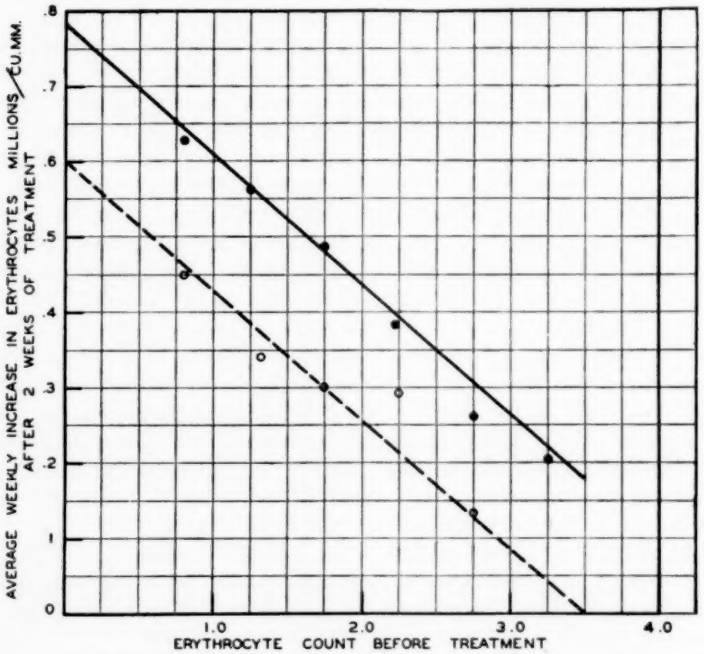

FIG. 1.—The relationship of the weekly increase in the erythrocyte count to the erythrocyte count before treatment in "satisfactorily" and "unsatisfactorily" treated patients with pernicious anemia after the first 2 weeks of treatment. *Solid dots:* average observed values of the weekly increase in the erythrocyte count with "satisfactory" treatment. *Solid line:* the average weekly increase in the erythrocyte count in "satisfactorily" treated patients, calculated from the equation $I = 0.78 - 0.174Eo$. *Open circles:* average observed values of the weekly increase in the erythrocyte count with "unsatisfactory" treatment. *Broken line:* the average weekly increase in the erythrocyte count in "unsatisfactorily" treated patients calculated from the equation $I = 0.60 - 0.176Eo$.

treatment, they must bear a direct relationship to the character or numbers of the erythrocytes present during relapse.

These equations, which are derived from data taken at the end of the first 2 weeks of treatment were found to be valid in all cases only for this period. The rise in the erythrocyte count is not uniform throughout the remission, being rapid at first and slower toward the end of the remission. In patients with low initial erythro-

cyte values this difference in the rate of erythrocyte increase in successive weeks is more noticeable than those with higher erythrocyte counts.

The equation I = 0.78 − 0.174Eo* is offered as a standard for the relative effectiveness of treatment of any type in patients with pernicious anemia. The expected average normal values as determined from this equation are tabulated in Table 2. This equation represents an erythrocyte response which will result eventually in an erythrocyte count of about 4.5 million per c.mm. of blood in from 6 to 8 weeks of treatment depending upon the level of the erythrocyte count before treatment.

Patients with an average weekly increase in the number of erythrocytes at the end of 2 weeks of treatment which is less than that expected from this equation will eventually have an erythrocyte count less than 4.5 million per c.mm. of blood, if the erythrocyte response continues at the same magnitude. Empirically, it has been determined from the data available that if the positive value in the equation is about 0.9 the eventual erythrocyte count will be approximately 5 million per c.mm., if it is 0.7 about 4.15 million per c.mm. of blood, if the dosage of the therapeutic material is not varied during the period involved. The end of the first 2 weeks of treatment is chosen as the most desirable time to estimate the average weekly increase in test patients. This test period of 2 weeks duplicates the conditions under which the standard values are derived. Beyond this 2-week period comparable values will not be obtained if the original erythrocyte count is low. Technical errors in the estimation of the erythrocyte counts before treatment and after treatment from which the increase in the erythrocyte count is estimated are minimized by choosing a period longer than 1 week.

If it is desired to compare the effect of a sample of some particular therapeutic material to the average effect of the doses of material of known potency which are customarily used, the equations which deal with the material in question can be used as standards. For example, a sample of intravenous liver extract of unknown potency is given in the usual dose of 20 cc. per week (prepared from 100 gm. of liver) to a patient with pernicious anemia whose initial erythrocyte count is 1 million per c.mm. of blood. The average weekly increase in the erythrocyte count is calculated at the end of the second week of treatment from observed erythrocyte counts and found to be 0.710 million per c.mm. of blood. The expected value from the equation I = 0.88 − 0.175Eo (Table 1, Equation 4) under these circumstances is 0.705. Thus the erythrocyte response is that which would be expected from the same dose of liver extract of average potency. If, however, the observed value was 0.500,

* Average weekly increase in erythrocyte count millions per c.mm., (I) equals 0.78 minus 0.174 times the initial erythrocyte count millions per c.mm. (Eo).

the tested extract obviously would be inferior in potency as compared to the standard intravenous liver extract.

Untoward Factors Which Affect the Interpretation of the Erythrocyte Response. In the interpretation of the reticulocyte response by means of the standard reticulocyte equations[1,3,4,6] in common use, many factors which may alter the magnitude of the reticulocyte response must be considered if the correct conclusions are to be reached. Most of these same factors also affect the erythrocyte response. Any alteration of the concentration of the erythrocytes in the blood as might result from hemorrhage, transfusion, or hemolysis unnatural to pernicious anemia alter the values of the erythrocyte increase. Any change in the fluid content of the blood, as by dehydration, likewise produces unsatisfactory results. Other disorders, present as complications of pernicious anemia, which affect erythrogenesis affect the erythrocyte response. Recent treatment makes the interpretation of the erythrocyte response unreliable. Unsuspected spontaneous remissions likewise may affect the reliability of interpretation of the results in some cases. Technical, mechanical and human errors in the estimation of the erythrocyte count are a constant source of error. The mathematical error inherent in measurements of any sort is a constant source of error in erythrocyte counts. Under the most scrupulous technique errors of this type of 200,000 cells per c.mm. are not unusual. Since erythrocyte counts are the basis of these equations the normal probability of such an error must be considered in the interpretation of results in individual cases. This error is lessened if the average of several estimations of the erythrocyte count for the value is used in the necessary computations.

The Relation of the Dose of Therapeutic Material to the Erythrocyte Response. On studying the erythrocyte response in this large number of patients with pernicious anemia, the magnitude of the erythrocyte response as measured by the average weekly increase in the concentration of the erythrocytes in the blood during the first 2 weeks of treatment is found to be directly in proportion to the effectiveness of treatment and the size of the effective dose of the material used. The determination of the exact nature of the latter relationship is impossible from the data at hand. The exact relationship between the therapeutic dose and the erythrocyte response can be found only under the rigid experimental conditions pointed out by Minot[2] in a consideration of the same problem in relation to the reticulocyte response.

Important among the conditions necessary in such an experiment are exclusion from the diet of sources of the extrinsic factor or of other sources of the pernicious anemia principle than the therapeutic material used; use of doses of the therapeutic material sufficiently small to produce an average or less than average erythrocyte response; elimination of test patients in whom factors are present

which alter the concentration of erythrocytes in the blood or alter erythrogenesis and the use of a single lot of therapeutic material of uniform potency in varying doses in patients with a similar degree of anemia.

Comparative Usefulness of the Reticulocyte Response and the Erythrocyte Response in Evaluating the Effectiveness of Treatment. The standard reticulocyte equations have been very useful in the standardization of liver extracts and in adjusting the therapeutic dosage to the needs of individual patients, although there has been some doubt expressed as to their value for these purposes.[5a,b] The use of the reticulocyte response in the evaluation of the effect of treatment in pernicious anemia has certain obvious practical disadvantages as compared with the use of the erythrocyte response. The erythrocyte response has similar disadvantages in this regard. With certain reservations, far more is known of the laws which govern the behavior of the reticulocytes in relation to treatment than is known regarding the rise in the erythrocyte count. Practically speaking, the most exact correlated data available for use in the literature for comparing the changes in the erythrocyte count in relation to the dosage of therapeutic material are found in the articles by Murphy.[5a,b] These particular data represent the increase in the erythrocyte count in a highly specialized form of treatment in which relatively large doses of liver extract are administered at infrequent intervals. They represent approximately the maximum erythrocyte response possible to obtain with large doses of liver extract. A rise in the erythrocyte count of similar magnitude was observed in a large proportion of the patients receiving intravenous liver extract in the present study. The eventual clinical improvement in these patients was no more satisfactory and the eventual level of the erythrocyte count was no greater than in many other patients who were given comparatively smaller doses of liver extract or ventriculin more uniformly during the period of remission.

Essentially the same situation exists in the interpretation of the reticulocyte response. As compared to values in the original standard reticulocyte curve designed for use in patients treated orally with liver extract[6] the average maximum reticulocyte percentages observed in patients treated with intravenous liver extract[1] are much greater. Nevertheless, the clinical improvement and the eventual level of the erythrocyte count were equally satisfactory under both types of treatment in adequately treated cases. The point of the matter is that a standard to be satisfactory should represent the minimum erythrocyte response which will result in the desired clinical and hematologic improvement.

The various standard reticulocyte equations[1,3,4,6] have counterparts in the various standard equations for the erythrocyte response set forth here. The equation $I = 0.78 - 0.174Eo$ which serves in general as a standard for the average erythrocyte increase under all

types of treatment and in particular for the erythrocyte increase with the customary treatment with liver extract orally (daily dosage of liver extract derived from 300 to 500 gm. of liver during early remission) and for the treatment with liver extract intramuscularly (in daily dosage of liver extract derived from 10 to 15 gm. of liver) has an almost identical significance with the standard equations for the reticulocyte response used in patients treated with oral liver extract ($EpR = 0.73 - 0.2Eo^3$ and $R = \frac{0.73 - 0.2Eo^6}{0.73 + 0.8Eo}$).

TABLE 2.—THE AVERAGE WEEKLY INCREASE (I) IN THE ERYTHROCYTE COUNT TO BE EXPECTED IN PATIENTS WITH PERNICIOUS ANEMIA AFTER 2 WEEKS OF ADEQUATE THERAPY AT VARIOUS LEVELS OF THE ERYTHROCYTE COUNT BEFORE TREATMENT (Eo).

Calculated from $I = 0.78 - 0.174Eo$.

Eo. Millions per c.mm.	I.	Eo. Millions per c.mm.	I.
0.5	0.693	2.5	0.345
0.6	0.676	2.6	0.328
0.7	0.658	2.7	0.310
0.8	0.641	2.8	0.293
0.9	0.623	2.9	0.275
1.0	0.606	3.0	0.258
1.1	0.589	3.1	0.241
1.2	0.571	3.2	0.223
1.3	0.554	3.3	0.206
1.4	0.536	3.4	0.188
1.5	0.519	3.5	0.171
1.6	0.502	3.6	0.154
1.7	0.484	3.7	0.136
1.8	0.467	3.8	0.119
1.9	0.449	3.9	0.101
2.0	0.432	4.0	0.084
2.1	0.415	4.1	0.067
2.2	0.397	4.2	0.049
2.3	0.380	4.3	0.032
2.4	0.362	4.4	0.014

The equation $I = 0.78 - 0.174Eo$ and $EpR = 0.73 - 0.2Eo^3$ have obvious similarities and were derived in a similar manner, by a comparison of averages of the observed data to the erythrocyte count before treatment.

In Table 3 the comparison is made between the relative dosage of ventriculin and liver extract administered in various ways; the observed average weekly increase in the erythrocyte count after the first 2 weeks of treatment and the values for the expected weekly increase in the erythrocyte count as calculated from the equation $I = 0.78 - 0.174Eo$. The observed and expected values of the increase in the erythrocyte count were approximately the same in the patients in this series treated with oral and intramuscular liver extract at all levels of the erythrocyte count before treatment. The observed values with ventriculin were usually considerably lower than the expected values. The observed values from the patients given intravenous liver extract in this series and

from the patients whose data are reported by Murphy[5a,b] are considerably higher than the expected average normal values calculated from the equation $I = 0.78 - 0.174Eo$. These differences in the observed and calculated values are obviously related to the weekly effective dose administered in each group. The high values observed in the patients receiving liver extract intravenously and in Murphy's patients do not necessarily indicate that the eventual erythrocyte count will be proportionately greater or that

TABLE 3.—COMPARISON OF THE INCREASE IN THE ERYTHROCYTE COUNTS IN PERNICIOUS ANEMIA AFTER THE FIRST 2 WEEKS OF TREATMENT UNDER VARIOUS TYPES OF THERAPY.

Type of treatment.	Number of cases.	Average weekly dose, gm.	Range of R.B.C. before treatment.	Average R.B.C. before treatment.	(I) Average weekly increase R.B.C., millions per c.mm. (observed).	Normal average weekly increase R.B.C., millions per c.mm. (calculated I = 0.78 − 0.174Eo).
Oral liver extract	12	3800*	0.5–1.0	0.79	0.693	0.643
	22	3800	1.0–1.5	1.28	0.560	0.557
	14	3350	1.5–2.0	1.77	0.434	0.472
	14	3400	2.0–2.5	2.21	0.427	0.396
	6	3500	2.5–3.0	2.70	0.280	0.310
	6	3400	3.0–3.5	3.39	0.182	0.191
Ventriculin	12	270	0.5–1.0	0.78	0.384	0.644
	23	255	1.0–1.5	1.25	0.490	0.562
	23	278	1.5–2.0	1.72	0.462	0.481
	22	244	2.0–2.5	2.17	0.364	0.402
	13	250	2.5–3.0	2.73	0.224	0.305
Intramuscular liver extract	4	85*	0.5–1.0	0.90	0.637	0.623
	12	98	1.0–1.5	1.26	0.581	0.561
	15	98	1.5–2.0	1.78	0.462	0.471
	13	80	2.0–2.5	2.19	0.315	0.399
	9	55	2.5–3.0	2.74	0.182	0.303
Intravenous liver extract	16	100*	0.5–1.0	0.82	0.721	0.635
	22	98	1.0–1.5	1.25	0.671	0.562
	27	98	1.5–2.0	1.69	0.581	0.490
	19	95	2.0–2.5	2.22	0.560	0.394
	19	105	2.5–3.0	2.67	0.357	0.315
	8	95	3.0–3.5	3.15	0.182	0.232
Intramuscular liver extract (Murphy[5a,b])	..	200*	1.0–1.5	1.23	0.895	0.570
	..	to	1.5–2.0	1.75	0.685	0.475
	..	300	2.0–2.5	2.26	0.625	0.387
	..	..	2.5–3.0	2.74	0.525	0.303
	..	..	3.0–3.5	3.34	0.490	0.299

* Liver extract in terms of grams of liver from which extract is derived.

the normal erythrocyte count will be achieved sooner than in the patients in the groups treated with oral and intramuscular liver extract. The average erythrocyte counts observed in these variously treated patients were practically the same (about 4.6 millions per c.mm.) at the end of the sixth week of treatment both in the patients reported by Murphy and those reported here. On the other hand, in the patients treated with ventriculin in whom the erythrocyte response at the end of 2 weeks of treatment was under

the expected normal value, the average erythrocyte count was about 3.9 million per c.mm. at the end of the sixth week of treatment.

Observed values of the average weekly increase in the erythrocyte count at the end of the second week of treatment equal to or higher than the values calculated from the equation $I = 0.78 - 0.174Eo$ indicate the return of the erythrocyte count to a normal level 4.5 to 5.5 million per c.mm., within 2 months of treatment, if adequate treatment is continued throughout the period. Observed values for the average weekly increase in erythrocytes at the end of 2 weeks of treatment distinctly under the calculated values signify inadequate treatment. In such cases, the eventual erythrocyte count will be distinctly less than normal unless the amount of treatment is increased.

When the standard reticulocyte equations[1,3,4,6] and the proposed standard equations of erythrocyte increase are used in the manner suggested here, in individual patients satisfactory for the purpose similar conclusions as to the adequacy or inadequacy of treatment will be arrived at by either method. Neither the reticulocyte response nor the erythrocyte response is superior for the purpose of estimating the effectiveness of treatment. The two methods should be used in conjunction with one another, one verifying the significance of the other.

Summary. 1. From data in 523 patients with pernicious anemia satisfactorily treated, the average weekly increase in the erythrocyte count at the end of 2 weeks of treatment was found to bear an inverse relationship to the erythrocyte count before treatment.

2. This relationship is expressed in the equation $I = 0.78 - 0.174Eo$ where I is the average weekly increase in the erythrocyte count after 2 weeks of treatment and Eo the erythrocyte count before treatment expressed as millions of erythrocytes per c.mm. of blood.

3. This equation is suggested as a standard for measuring the relative effectiveness of treatment in pernicious anemia.

4. Observed values equal to or greater than those obtained from this equation indicate adequate treatment. Observed values less than those calculated from the equation indicate inadequate treatment.

5. The existence of various complicating factors such as concurrent disease, transfusion or hemorrhage in association with pernicious anemia, invalidate the use of this standard.

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THE BLOOD OF NEWBORN RATS AFTER ORAL ADMINISTRATION TO THE MOTHER OF NORMAL AND ABNORMAL HUMAN GASTRIC JUICE.*

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One of the more conspicuous gaps in our knowledge of pernicious anemia is the lack of any reliable, non-clinical, laboratory test for the detection of antianemic factor and for the determination of the potency of therapeutic antianemic preparations. In spite of considerable work on the subject there is still no accredited test or assay technique which does not require the use of relapsed pernicious anemia patients. Since there is no condition in animals comparable to true pernicious anemia in man, investigation has been limited to the effects of the administration of antianemic substances to normal animals or to animals with artificially induced anemia, and to certain biochemical tests.

Studies on normal animals have been concerned chiefly with attempts to alter the number of circulating reticulocytes. Investigations of this type led to the "pigeon test" of Vaughan, Muller and Zetzel,[17] the "guinea-pig test" of Jacobson[10] and the "rat reticulocyte reaction" or "R. R. R. test" of Singer.[14] The blood of dogs, rabbits, pigs and sheep also has been investigated from this standpoint.

Experimental anemia has been induced in animals by the injection of various toxins and bacteria, by feeding deficient diets and by extensive bleeding. The reports which have appeared regarding any specific value of antianemic substance either in preventing or in remedying induced anemia are most conflicting. The earliest biochemical tests concerned the supposed property of antianemic extracts of forming methemoglobin when added to a suspension of washed erythrocytes.[7] More recently the enzymic properties of the "intrinsic factor" as a proteolytic agent have been investigated.[8,12,16] In general, laboratory methods have not yielded uniform or satisfactory results. Each test has its advocates, but widespread confirmation of any one has not been forthcoming.

A recent approach to the problem was an attempt to influence the rate of maturation of the blood in the developing fetus. The resemblance between the cells of fetal blood and those of the blood of patients with pernicious anemia has long been recognized, but Wintrobe and Shumacker[22] were the first to point out the similarity between the changes which occur in fetal blood as development proceeds and the changes occurring in the blood of an adequately treated patient with pernicious anemia. They suggested that the

* This work was done in the Division of Experimental Medicine, The Mayo Foundation.

same stimulating influence might be responsible for the changes which take place in both conditions. If this were the case, it should be possible to hasten the maturation of fetal blood by making available to the fetus an excess of antianemic factor, on the assumption that delayed maturation was due to a relative deficit of the maturation factor. It has been demonstrated of course that fetal tissues are not wholly devoid of the antianemic principle.[19]

The first attempt to influence fetal hemopoiesis was made by Wintrobe and his coworkers in 1937.[21] They were unable to alter the blood picture of fetal rabbits by the intramuscular injection of liver extract into the mother during pregnancy or by injection directly into the placenta at laparotomy. Stasney, Higgins and Mann[15] reported an increase in the number of circulating erythrocytes and a decrease in erythrocyte volume and diameter in the blood of newborn rats whose mothers had received daily intraperitoneal injections of concentrated gastric juice from normal human beings or from the fundic pouch in a hog. The effect of the gastric juice was proportional to the number of injections the mother had received. Heating the gastric juice prior to injection completely eliminated the effect on fetal blood. This led Stasney and his coworkers to conclude that normal human and swine gastric juice contained a substance capable of accelerating erythrocyte maturation in fetal rats.

Briese and Higgins[2] reported reductions in the diameters of erythrocytes of newborn rats whose mothers were fed ventriculin during pregnancy. These findings have been held to confirm the hypothesis that the physiologic macrocytosis of the mammalian fetus is due to a lack of adequate amounts of the antianemic principle. By still further reducing the amount of antianemic principle available to the fetus by damaging the liver of the mother by carbon tetrachloride inhalation, Briese[1] concluded that she was able to exaggerate the macrocytic anemia of newborn rats. Jones[11] studied blood smears prepared from the yolk sac of the 11-day rat embryo following parenteral injection of liver extract into the mother during pregnancy and observed a decrease of mean cell diameter and still greater decrease of mean nuclear diameter. These changes, along with the increased polychromatophilia, pyknosis and karyorrhexis which he noted, Jones interpreted as indicating an acceleration of cytoplasmic differentiation and nuclear maturation produced by the transmission of a superabundance of antianemic principle across the placenta.

Wigodsky and Ivy[18] were unable to detect any change in the blood of newborn rats to whose mothers they administered liver extract intraperitoneally during pregnancy. Bruner[3] reported similar negative results after the intramuscular injection of liver extract into pregnant rats. Reimer[13] was unable to detect any changes in the blood, liver or bone marrow of chick embryos after injecting

liver extract into the whites of intact eggs. Injections into the allantois were also performed, but no difference was noted between the treated embryos and untreated controls.

The study of the factors concerned with fetal hemopoiesis only recently has arrived at the experimental stage. A great deal of work will have to be done before any conclusions can be drawn regarding the possible identity of factors operative in the embryo and curative in pernicious anemia. Aside from the academic value of such studies, it is apparent that, if it can be demonstrated that fetal hemopoiesis can be accelerated by antianemic preparations, then a whole new field lies open for the further investigation of methods for detecting the presence of antianemic factors and testing the potency of materials containing them.

Methods. Samples of human gastric juice, collected after ingestion of a test meal of arrowroot biscuits and water, were obtained from the gastroenterologic laboratory. These were filtered, pooled in several different lots and refrigerated until needed. The first lot was composed of specimens considered to represent normal gastric secretion, containing 20° to 40° of free hydrochloric acid (Töpfer's method). This material was administered to 6 pregnant albino rats by gastric intubation in daily doses of 1 to 3 cc. during the last 2 weeks of gestation. Early pregnancy was determined by daily vaginal smears. Seven pregnant rats received normal gastric juice which had been heated over boiling water for 20 minutes, and 6 pregnant rats received normal gastric juice which was neutralized with concentrated sodium hydroxide immediately preceding administration. Gastric juice lacking free hydrochloric acid, obtained from patients with functional dyspepsia, hypochromic microcytic anemia, gall bladder disease, and other conditions, was given to 9 pregnant rats. Six pregnant rats received gastric juice obtained from patients having pernicious anemia and 3 pregnant rats were treated with gastric juice from patients suffering with carcinoma of the stomach. Thirteen untreated pregnant rats served as controls. An abundant supply of the normal laboratory ration (23.4% protein content) was constantly available to all rats. The total of 50 rats gave birth to 362 offspring.

Samples of blood were obtained from the newborn rats by cardiac puncture within a few hours after birth. The total number of erythrocytes per cubic millimeter of blood was determined by counting two samples from a standard pipet in a Neubauer ruled counting chamber. The volume of packed red cells per 100 cc. of blood was determined with the aid of van Allen hematocrit tubes. The mean corpuscular volume was calculated according to the method of Wintrobe.[20] Heparin was used as the anticoagulant. The mean erythrocyte diameter was determined by direct measurement of 50 cells on photographs of dry smears stained with Wright's stain and magnified 1500 times. Fields in which the blood cells were distorted or overlapping one another were avoided, and the attempt was made to measure only round cells. When a cell displayed only a slight tendency to oval shape, the greater diameter of the cell was measured. Stained smears were studied for the presence of nucleated erythrocytes and the number observed in counting 2000 erythrocytes was noted. The percentage of reticulocytes present was determined by recording the number observed while counting 500 erythrocytes on smears made after mixing a drop of blood with aqueous brilliant cresyl blue and counterstaining with Wright's stain. Probable errors were computed for all values determined.

The author wishes to express his appreciation to Dr. G. M. Higgins for his generous advice, assistance and encouragement in the carrying out of this work and the preparation of this paper, and to Miss Catherine Sawyer for her kind coöperation in making available samples of gastric juice.

Results. The blood values of the 362 newborn rats are shown (Table 1). It will be seen that the average values of the blood of offspring of mothers which received heated normal gastric juice or gastric juice of patients having pernicious anemia were quite similar to such values of the blood of offspring born to untreated mothers. The mean values in the blood of offspring of mothers which received normal or neutralized normal gastric juice or gastric juice removed from patients with carcinoma differ significantly from the values just mentioned. The total number of erythrocytes of this group exceeds 3,000,000 per cubic millimeter. The hematocrit values remaining unchanged, the mean corpuscular volumes were lowered to the vicinity of 145 cubic microns. The mean cell diameters were smaller by 0.5μ or more than those of the other group. In between the values of these two groups were the values observed on offspring whose mothers had received the achlorhydric gastric juice. The percentage of reticulated and of nucleated cells present was subject to considerable variation in all groups and showed no essential relation to any of the other changes noted.

TABLE 1.—MEAN BLOOD VALUES OF NEWBORN RATS.

Type of gastric juice administered to mother during pregnancy.	No. of newborn rats.	Erythrocytes, millions per c.mm.	Packed red cells, cc. per 100 cc.	Corpuscular volume, cu.μ	Reticulocytes, %	Normoblasts, %	Erythrocyte diameter, μ.
None (untreated controls)	92	2.93±0.02	45.1±0.24	153.6±0.98	88.9±0.69	0.22±0.01	9.73±0.02
Heated normal gastric juice	51	2.83±0.03	43.7±0.20	154.6±0.85	91.7±0.74	0.20±0.01	9.60±0.03
Gastric juice from patients having pernicious anemia	43	2.83±0.03	44.5±0.46	157.3±1.03	91.9±0.74	0.22±0.02	9.60±0.04
Normal gastric juice	49	3.15±0.04	45.7±0.37	145.1±1.21	89.6±0.84	0.20±0.02	9.13±0.03
Neutralized normal gastric juice	43	3.24±0.03	46.5±0.38	143.9±1.11	95.1±0.57	0.21±0.02	8.94±0.03
Achlorhydric gastric juice	62	3.00±0.03	43.6±0.32	146.2±1.10	86.7±1.14	0.24±0.01	9.19±0.05
Gastric juice from patients having gastric carcinoma	22	3.41±0.05	48.0±0.57	141.1±1.72	91.4±1.33	0.14±0.01	9.07±0.05

Although the differences in the mean cell diameters of the various groups were definite, these figures give but an incomplete picture of the actual changes which were observed in the erythrocytes as seen in dry smears. A more complete and graphic basis for comparison is obtained by the construction of Price-Jones curves. In Chart 1 such graphs for the blood of newborn rats whose mothers had received normal and heated normal gastric juice are compared with a similar curve of the newborn of untreated controls. The curve depicting the distribution in the "heated" group is essentially identical to that of the "control" group, whereas the curve depicting changes induced by the normal gastric juice is shifted to the left toward the smaller diameters.

Percentage distribution

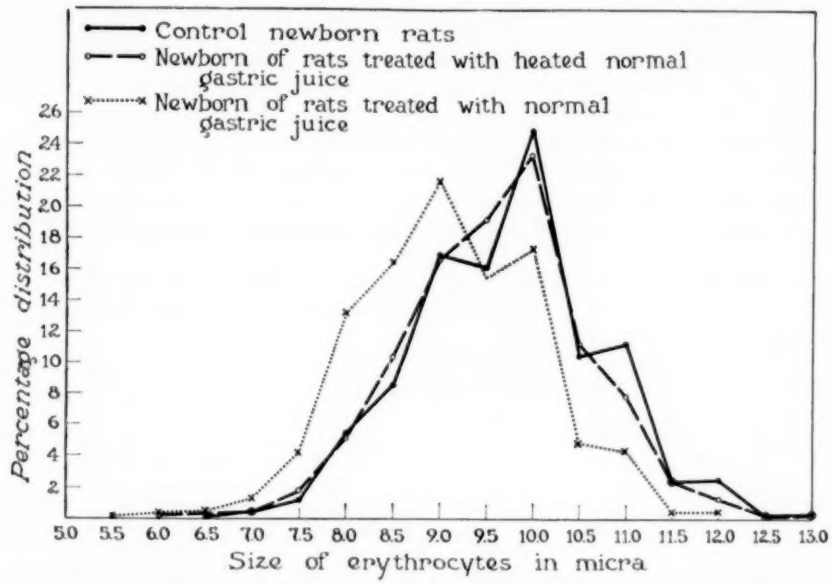

CHART 1.—Mean Price-Jones curve for blood of group of newborn rats whose mothers were treated with heated normal gastric juice and of group whose mothers received normal gastric juice compared with mean curve of control group of newborn rats whose mothers received no treatment during pregnancy.

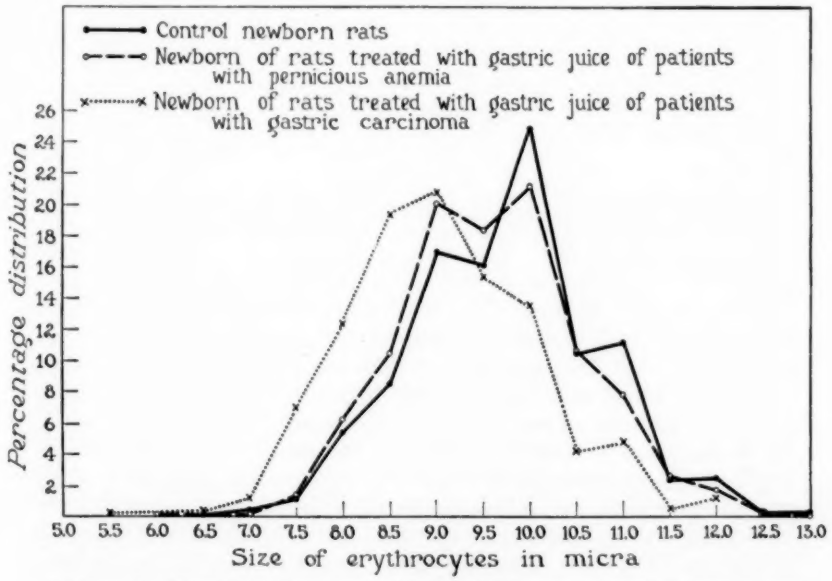

CHART 2.—Mean Price-Jones curve for blood of group of newborn rats whose mothers were treated with gastric juice of patients having pernicious anemia and of group whose mothers received gastric juice from patients having carcinoma of the stomach compared with mean curve of control group

In Chart 2 the curve of distribution of red cell diameters of the newborn of mothers receiving the pernicious anemia gastric juice is quite similar to that of the control group; whereas a similar curve for the carcinoma gastric juice lies considerably to the left, in the direction of smaller red cell diameters. The curve for the neutralized normal gastric juice was quite far to the left of that showing the control distribution (Chart 3); whereas the curve for the achlorhydric gastric juice occupies a position intermediate between the two.

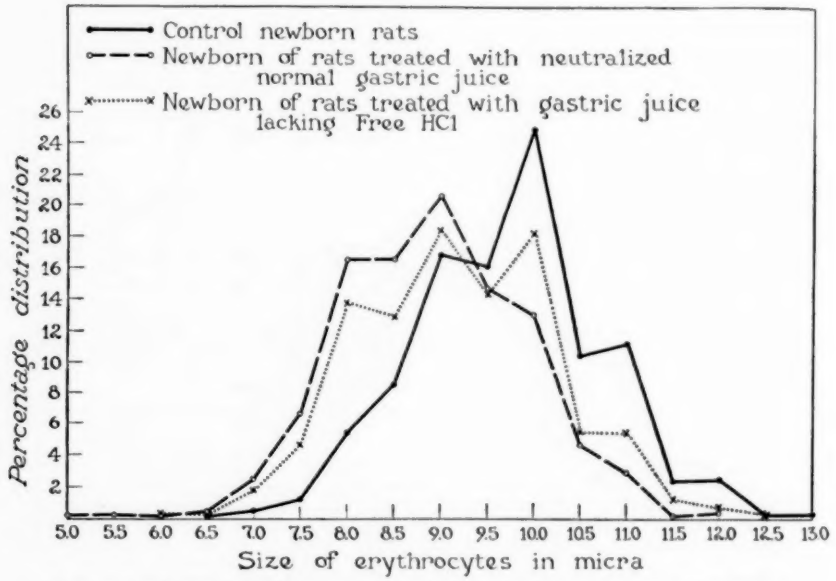

CHART 3.—Mean Price-Jones curve for blood of group of newborn rats whose mothers were treated with neutralized normal gastric juice and of group whose mothers received gastric juice from patients with achlorhydria compared with mean curve of control group.

These relationships are numerically expressed in Table 2. Here the range of erythrocyte diameter of the controls, 6.5 to 13μ, is divided into three approximately equal divisions. The percentage of all cells falling into each of these three divisions is indicated for each of the various groups of animals studied. It will be noted in the table that the relative proportion of smaller cells is very much higher in the groups of newborn whose mothers had received normal or neutralized gastric juice, or gastric juice of patients with carcinoma of the stomach or simple achlorhydria, than in those constituting either the "control," the "heated" or the "pernicious anemia" group.

When individual determinations, rather than means, are considered, marked variations and overlappings of the data are noted for

all the values. Variations occur not only between individuals in different groups but between individuals in the same group and even in the same litter. Moreover, even means of litters within the same group exhibit variations and it is only when the means for a large number of litters (or individuals) are considered that trends can be definitely discerned. No correlation could be detected between any blood values of the newborn and the size of litters or the degree of maternal anemia.

TABLE 2.—DISTRIBUTION OF ERYTHROCYTES ACCORDING TO DIAMETERS.

(Control Range = 6.50μ to 13.00μ, divided into three approximately equal divisions.)

Type of gastric juice administered to mother.	% measuring less than 8.50μ.	% measuring between 8.50μ and 10.50μ.	% measuring more than 10.50μ.
None (untreated)	7.15	76.81	16.04
Heated normal gastric juice	7.73	80.47	11.80
Gastric juice of patients having pernicious anemia	8.00	78.54	13.46
Normal gastric juice	18.88	75.65	5.47
Gastric juice of patients with gastric carcinoma	20.72	72.63	6.65
Achlorhydric gastric juice	20.87	71.55	7.58
Neutralized normal gastric juice	26.48	69.91	3.61

Comment. In considering the differences in the assembled data of the blood of newborn rats whose mothers had received the various substances, two questions arise: 1, Are the changes significant? 2, What explanation may be found for these changes?

The answer to the first question depends on a number of factors. Since the differences noted were small, the first consideration must be the accuracy of the methods employed in arriving at the values recorded. Certainly the differences are too small to exceed the limits of experimental errors and individual variation in a single, or small number of determinations, even when experience, care in technique and consistency of method have reduced error to a minimum. Only on the basis of a large number of observations can these differences assume any importance. Since all but one of the groups of newborn rats, from which blood samples were taken, contained between 40 and 90 animals, it would seem that the requirement in numbers necessary to establish the significance of the results has been met. It must be understood that the changes reported here represent variations in means of recorded data, thereby indicating general trends, but do not represent changes which consistently may be reproduced to an equal degree in any individual.

Stasney, Higgins and Mann[15] expressed the opinion that the increase in the number of circulating erythrocytes associated with a decrease in their volumes and diameters observed in the blood of newborn rats whose mothers had received normal human or swine gastric juice intraperitoneally represented an acceleration of erythrocyte maturation which was induced by some substance present

in the concentrated gastric juice. Jones emphasized the fact that the changes in fetal blood which he observed in his studies did not indicate transformation of primitive erythroblasts of the prehepatic period into normoblasts or definitive red blood cells, but a speeding of their maturation toward primitive erythrocytes. It is generally accepted that such changes, occurring in the blood of a patient with pernicious anemia following the oral administration of normal gastric juice (with suitable opportunity for contact with a source of "extrinsic factor"), represent an acceleration of maturation. Restoration of normal red cell maturation then follows. Whether these changes observed in the blood of the rat fetus are at all comparable and the result of an availability of larger supplies of antianemic material provided by the administration of gastric juice to the mother cannot definitely be stated.

The administration of gastric juice by intubation was considered a more physiologic method than administration by injection. If the changes described by Stasney and his coworkers were due to the antianemic factor in gastric juice, such changes should also appear when the materials were given orally, providing of course that ample opportunity was afforded for contact with an abundant source of "extrinsic factor." This opportunity was provided and changes in the blood similar to those which Stasney and his coworkers described were obtained.

If one accepts the explanation offered by Stasney and his coworkers and the hypothesis of Castle,[4,5] a ready hypothesis is then at hand to explain these results. Normal gastric juice provides an excess of "intrinsic factor" which after contact with "extrinsic factor" of the diet may result in the production of an excess of antianemic principle in the pregnant rat. This would overcome the relative deficiency of this principle resulting from the burden of fetal hemopoiesis. Accordingly, more of the antianemic material would be available to the fetus and fetal hemopoiesis would be thereby enhanced. As the criterion of increased maturity we find in the blood of the newborn whose mothers received normal gastric juice a 7.51% increase in the number of circulating erythrocytes, a 5.53% reduction in the mean corpuscular volume, and a 6.16% decrease in the mean erythrocyte diameter.

The material in gastric juice which produces these changes in the blood of newborn rats bears resemblance to the "intrinsic factor" in some of its properties and distribution. The effectiveness of both is destroyed by heating. Castle and his coworkers[6] concluded that an acid environment inhibited the activity of the "intrinsic factor." Neutralization of the normal juice here employed seemed to enhance its activity. "Intrinsic factor" is lacking from the gastric juice of patients with pernicious anemia, or at least greatly reduced in amount. Gastric juice obtained from such patients was without effect on the blood picture of newborn rats.

The variable results obtained with gastric juice from patients with simple achlorhydria and gastric carcinoma are comparable to the results obtained by Hartfall and Witts[9] in their clinical studies with achlorhydric gastric juice. They concluded that the amount of antianemic material present in achlorhydric juice was always less than normal and subject to considerable variation. Several of the litters born to rats given such gastric juice samples showed no effect on the fetal blood cells.

Whether the material which produced the changes herein described is the "intrinsic factor," or whether the same factors govern hemopoiesis in the rat as in man, cannot be ascertained from the work at hand. In fact it has not even been established that these changes represent accelerated maturation. The various treatments administered were entirely without effect on the relative number of reticulated and nucleated red cells. Accordingly, if one thinks of maturation in terms of individual cells, there has been no progression of the cell toward a more mature type. The increase in number and decrease in volume and diameter would then have to be explained as the result of the premature appearance of later generations of erythrocytes, presumably from the fetal bone marrow which had been subjected to stimulation. This, of course, is actually maturation in a broader sense.

Summary. The normal blood values of the newborn albino rat have been determined for the conditions and technique of this study. Normal human gastric juice has been found to contain a substance which when administered orally to pregnant albino rats produces in the blood of the fetus a significant increase in the number of circulating erythrocytes and a decrease in their volume and diameter which can be detected at birth. This substance is without effect on the relative number of reticulated or nucleated red cells in the blood of the fetus. Its activity is enhanced by neutralization and destroyed by heating. The substance is absent from the gastric juice of patients having pernicious anemia. It is present in variable amounts in the gastric juice of patients with simple achlorhydria or carcinoma of the stomach. The possible relation of this substance to the intrinsic factor of Castle has been considered.

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HEMOLYSINIC ANEMIA AND HEPATIC DEGENERATION CURED BY SPLENECTOMY.

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A case of hemolysinic anemia showing clinical, metabolic and histopathologic evidences of impaired liver function is presented. A hemolysin was demonstrable in the blood serum prior to splenectomy, which was performed as an emergency measure because blood transfusions had aggravated the hemolysis and the liver dysfunction. Restoration of liver function and health and disappearance of the hemolysin followed the operation.

Case Report. P. A., a 17-year-old Jewish boy, was admitted to the Medical Service on April 18, 1939, and discharged on May 29, 1939.

Present Illness. The patient was in good health until March of 1937 when he was seized with chills and began to perspire profusely. The following morning jaundice of the skin and scleræ was noticed which became progressively deeper for 2 weeks and was associated with weakness, malaise, severe anorexia (especially for meat), nausea and occasional vomiting of bile-stained material. His skin did not itch; his urine was of dark color and his feces of normal, dark color. The jaundice gradually subsided; the patient returned to school and participated in athletics. However, examination at the Northern Liberties Hospital in May, 1937, showed the following: slight icterus, mild fever, slight leukocytosis and elevation of the icterus index. Roentgen ray examination at this time revealed a large gall bladder which emptied well after a fat meal without evidence of stones and a normal gastro-intestinal tract except for an irritable colon.

In August, 1938, an episode similar to that of March, 1937, occurred which lasted for 3 weeks. On April 1, 1939, weakness, malaise, anorexia and jaundice developed.

Past and Family History. The patient's general health had been excellent except for measles, pertussis and a myringotomy for otitis media in childhood. No other instances of jaundice appear in the family history.

Physical Examination. Temperature 100° F., pulse 96, blood pressure 130 systolic and 88 diastolic, weight 147 pounds, height 67¾ inches. The

patient was comfortable. The skin and visible mucosal surfaces were intensely icteric and pale. A soft systolic murmur was heard over the entire precordium. The liver edge could not be palpated. The rounded, firm, smooth edge of the spleen extended 6 cm. below the costal margin in the left anterior axillary line and moved with respiration. No abnormalities were observed in the ocular fundi or in the remainder of the physical examination.

Laboratory and Special Examinations. Hemoglobin, 5.8 gm. (Haden-Hauser) per 100 cc.; erythrocytes 2,450,000 per c.mm.; leukocytes 8150; differential count—neutrophils 62% (non-filamented forms 9%, filamented 53%), lymphocytes 33%, monocytes 5%, normoblasts, 6 per 100 leukocytes; reticulocytes 4.3%; platelets 197,000 per c.mm.; icterus index 80 units, serum bilirubin 7.5 mg. per 100 cc., van den Bergh reaction indirect; bleeding time, 1 minute, coagulation time, 3½ minutes, prothrombin time (Howell's method), normal; fragility test—0.44% to 0.36% sodium chloride solution (identical with the control test); mean corpuscular diameter—8.02 microns with the following distribution: less than 7.5 microns, 19.4%, equal to 7.5 microns, 32.2%, greater than 7.5 microns, 48.4%. The erythrocytes varied in size from 3.75 to 12 microns and the Price-Jones curve was a biapical type with peaks at 7.5 and 9 microns. These measurements were repeated with similar results and the erythrocyte fragility was normal on two other occasions. On the stained blood film, the red blood cells showed marked anisocytosis, poikilocytosis and polychromatophilia, but small, darkly-stained erythrocytes (microspherocytes) were not seen. The blood Wassermann and Kahn reactions, fasting sugar, urea nitrogen, chlorides, serum protein (and albumin/globulin ratio), phosphatase, cholesterol and cholesterol esters (Table 1) were all within normal limits. The

TABLE 1.—CHEMICAL VALUES BEFORE AND AFTER SPLENECTOMY.

Date, 1939:	April.		May.				
	20.	22–26.	3.	5.	8.	23.	22–26.
Serum cholesterol:							
Total, mg./100 cc.	122	...		122	..	104	
Esters, mg./100 cc.	68	...		34	..	61	
Esters, % of total	56	...		27	..	59	
Urobilinogen excretion, mg./24 hrs.:							
Feces	..	502.0		..	..	..	51.9
Urine	..	4.84		..	..	..	1.9
Serum protein, gm./100 cc.	7.0	...	SPLENECTOMY	..	5.1	6.2	

urine contained 4.84 mg. of urobilinogen per 24 hours[25a] but no bile pigment, bile acid salts, albumin, sugar, casts or red blood cells. The fecal urobilinogen* excretion averaged 502 mg. per 24 hours on the analysis of a 4-day stool specimen. Repeated aërobic and anaërobic blood cultures were sterile. The blood group was B (Type III Moss). The stools contained no fresh or occult blood, ova or parasites. No malarial or other parasites were ever seen in the blood films. The electrocardiogram was normal. Biliary drainage, with two magnesium sulphate stimulations, revealed: B bile, 30 cc.; B-C bile, 140 cc.; microscopically, cholesterin crystals, black pigment and shaggy mucus strands. The fasting gastric juice contained

* Urobilinogen determinations were conducted by R. H. Hamilton, Ph.D., M.D., of our Department of Biochemistry.

15 degrees of free acid. Radiographs of the skeleton revealed none of the changes occasionally observed[3] in the chronic hemolytic anemias and stones were not visualized in a flat plate of the abdomen.

Clinical Course. During the first week in the hospital the maximum daily temperature remained at 100° F. and the patient was comfortable. Since microspherocytosis and decreased resistance of the erythrocytes to hypotonic saline solution were not found, in a patient whose large family had no history of icterus, treatment with repeated blood transfusions seemed indicated rather than splenectomy.[24] Before transfusions were started the temperature began to increase and marked weakness, severe anorexia and vomiting developed. The course of the temperature, blood hemoglobin, red blood cell and serum bilirubin levels is shown in Chart 1. About 2000 cc.

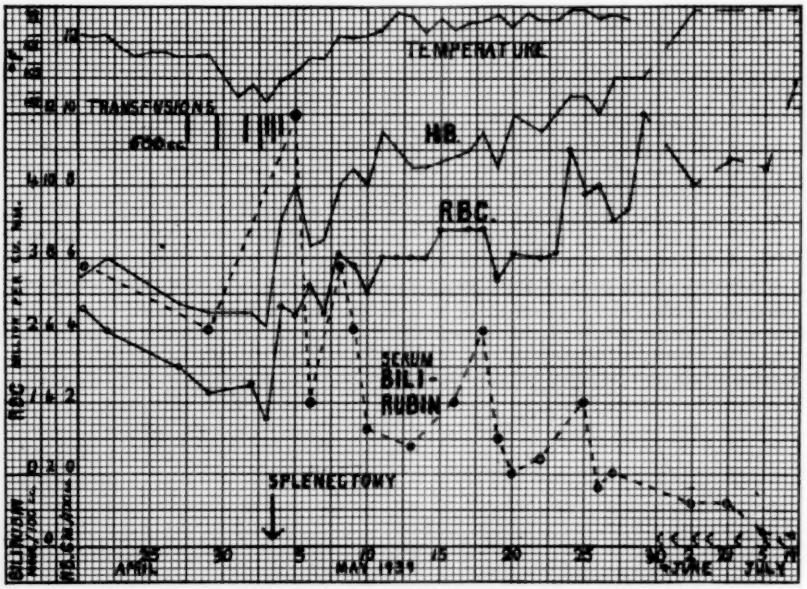

CHART 1.—Course of temperature, hemoglobin and red blood cell levels and serum bilirubin concentration. On the bilirubin curve the large, open circles indicate the indirect type of van den Bergh reaction; the large dots represent the direct type of reaction. The dates and the amounts of the transfusions are indicated.

of group B citrated blood was administrated during a 5-day period (Chart 1) without untoward reaction except for a non-productive cough associated only with the first two transfusions. The cells and serum of the several donors were carefully cross-matched with the patient's cells and serum and with each other's cells and serum.

The progressive decrease in the erythrocyte and hemoglobin levels and the increasing icterus index indicated that the rate of hemolysis was increasing rapidly. On the morning of May 3d, the patient was intensely jaundiced with an icterus index of 200 units; the red blood cell count had decreased to 780,000 per c. mm.; the rectal temperature was 105° F.; the pulse rate was 160 per minute; the blood pressure was 90 systolic and 50 diastolic; the patient was delirious and unable to retain anything by mouth. The liver edge was palpable 2.5 cm. below the costal cage in the right mid-

clavicular line while the spleen remained at 6 cm. Multiple retinal hemorrhages had appeared. Bilirubin and albumin were found in the urine but there were no casts or erythrocytes in the sediment and tests for acetone and diacetic acid were negative. There was no significant acidosis (blood carbon dioxide combining power 49 vol. %) or nitrogen retention (blood nonprotein nitrogen 29 mg. per 100 cc.).

In view of the failure to respond to transfusions and the desperate nature of the hemolytic crisis, emergency splenectomy offered the only hope of survival.[10] Under spinal anesthesia with 170 mg. of procaine, the spleen was quickly removed and a piece of the liver was excised for a biopsy (operation by W. E. B.). The spleen was enlarged and congested; the vessels of the pedicle were only slightly larger than usual; no adhesions were found. The liver extended 5 cm. below the costal margin and was of normal consistency and brown color with a slight bile stain. The gall bladder showed no thickening, emptied well on pressure and contained a stone about 2 to 3 mm. in diameter which was not removed. Within 2 minutes following ligation of the splenic pedicle, the systolic blood pressure rose from 90 to 120 and the pulse rate fell from 170 to 130 with a marked improvement in the volume of the pulse. Epinephrine was not employed either before or during the operation. A transfusion of 300 cc. of citrated Group B blood was given during the operation and an additional 700 cc. during the ensuing night using the same donors employed for the first two transfusions; no more transfusions have been given. The immediate postoperative condition was remarkably better than the preoperative state.

The postoperative course was clinically uneventful, except for blurred vision, related to the retinal hemorrhages, and for edema, associated with intravenous saline and glucose infusions and a decrease of the total blood serum protein level to 5.1 gm. per 100 cc. Postoperatively, the blood showed marked erythrocyte regeneration—increased macrocytosis (mean corpuscular diameter 8.8 microns on the fifth day), nucleated red blood cells (maximum of 31,000 per c.mm. on the third day), macro- and micro-normoblasts in mitosis (no megaloblasts), Howell-Jolly bodies and nuclear extrusion bodies—associated with neutropenia (minimum of 1180 filamented neutrophils per c.mm. on the second day), myelocytes (maximum of 87 per c.mm. on the fourth day) and thrombocytopenia (minimum of 58,000 per c.mm. on the sixth day). The temperature, pulse, symptomatology, erythrocyte count, hemoglobin level and icterus improved rapidly (Chart 1). The effect of supportive therapy during the critical pre- and postoperative days with intravenous glucose and physiologic saline solutions, insulin, thiamin chloride, liver extract (pernicious anemia type), yellow bone marrow extract, vitamin K (Klotogen), bile acid salts, dilaudid and prostigmine cannot be evaluated.

Less than 4 weeks after splenectomy, the patient was able to walk about all day without fatigue; the icterus had almost entirely disappeared and there was no pallor. The retinal hemorrhages were absorbing. Body weight had decreased to 131 pounds. Special laboratory studies repeated during the last week in the hospital showed: fecal urobilinogen 51.9 mg. per 24 hours; urinary urobilinogen 1.9 mg. per 24 hours; normal serum cholesterol, cholesterol esters and total protein levels (Table 1); normal blood urea nitrogen concentration; no occult blood in the urine; no hemoglobinuria (spectroscopically); in the biliary drainage: 40 cc. of B bile, 120 cc. of C bile, macroscopic amounts of calcium bilirubin pigment, but no cholesterin crystals microscopically; in the Roentgen ray examination of the gall bladder with tetraiodophenolphthalein: poor concentration of the dye but good contraction after the fat meal and no evidences of stones.

Eleven weeks after the splenectomy the patient reported that he had been living an entirely normal life and was gaining weight (160 pounds).

Icterus of the scleræ was no longer detectable; the liver edge was just palpable at the costal margin on deep inspiration; there were no abnormal physical findings except the absorbing retinal hemorrhages. Examination of the blood showed: erythrocytes 5.45 million per c.mm.; hemoglobin 13 gm. per 100 cc.; mean corpuscular diameter 7.9 microns with a range of 4.5 to 10.5 microns and the following distribution: less than 7.5 microns, 12% equal to 7.5 microns, 40.4%, greater than 7.5 microns, 47.6% and a monoapical Price-Jones curve; leukocytes 7700 per c.mm. with a relative lymphocytosis in the differential leukocyte count. Four months after the operation and after a camping trip in the mountains the patient was in fine condition, he was no longer conscious of blurred vision; the blood hemoglobin was 16.5 gm. per 100 cc. and the erythrocyte count was 5.64 million per c. mm.

Pathologic Anatomy.* The spleen measures 22 by 15 by 12 cm. and weighs 1000 gm.; its capsule is smooth and rather tense; it is firm; the follicles are not distinguishable. Microscopically (Fig. 1) there is a remarkable hyperplasia of the reticulum; numerous areas are made up entirely of reticulum cells with the exclusion of lymphocytes. There is much evidence of a rapid red blood cell destruction, most of the red cells showing a vague, smudged outline and there is considerable fresh and old blood pigment. Erythrophagocytosis is not seen. There is a diffuse infiltration with neutrophils. The lymphoid follicles are few in number, rather poorly demarcated and show an underdevelopment of the secondary follicles. The sinuses are not abnormal. The specially stained contact preparations of the spleen show young forms of both erythrocytes and granulocytes indicating myelogenous metaplasia. Thus the picture is one of reticulum hyperplasia and hemopoiesis of the spleen with increased red cell destruction.

It should be emphasized that this histopathology is not characteristic of the spleen in congenital hemolytic jaundice[16] but resembles that reported by Israëls and Wilkinson[14] in 4 adults with acholuric jaundice.

Microscopic examination of the liver biopsy (Fig. 2) reveals a very marked fatty change. Well over one-half of the cells show huge vacuoles with destruction of almost the entire cell. The remaining cells show small globules of fat, bile pigment retention, and granular degeneration of the cytoplasm, the whole picture being one of degeneration. The portal areas show a striking infiltration with neutrophils. These take no special arrangement but are diffusely scattered throughout the portal areas. The majority of the biliary radicles are healthy in appearance.

Serology. A hemolysin was demonstrated *in vitro* in the patient's blood serum before splenectomy. In preliminary tests (B. M. West, B.S., Chief Technician), 3 cc. of the patient's fresh blood serum (April 25th) were mixed with 3 drops of a 10% suspension of the patient's unwashed erythrocytes in physiologic saline solution, as well as with similar suspensions representing each of the four normal blood groups, and incubated in open tubes at 37° C. over night (14 hours). Complete hemolysis of the patient's Group B cells, the specimen of normal Group B cells and partial hemolysis of the normal Group O cells occurred. The normal Group A and AB cells showed the agglutination to be expected in the patient's Group B serum. Samples of normal serum of each of the four blood groups were incubated in a similar manner with the patient's cells; no hemolysis occurred; agglutination appeared in the Group A and O sera. On April 27th, just before the first transfusion, fresh samples of the patient's serum and cells gave identical results. Autoagglutination of the patient's cells in his own serum was not observed but the hemolysis that always occurred may have masked the recognition of macroscopic agglutination. When a tube of the patient's coagulated blood (serum and clot in contact) was incubated, hemolysis was not detected macroscopically.

* By E. E. Aegerter, M.D., of our Department of Pathology.

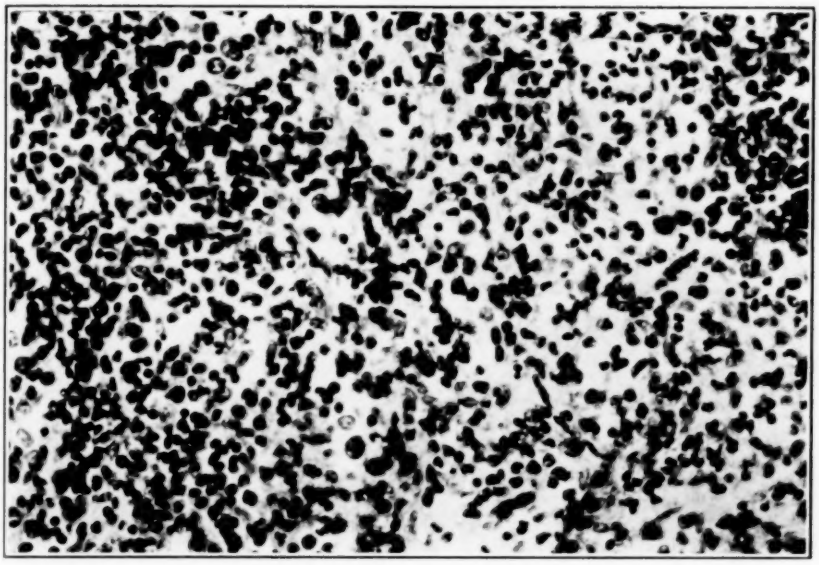

FIG. 1.—A section of the spleen with reticulum cell hyperplasia. There are numerous young forms of both red cells and myelogenous white cells. There is considerable old blood pigment. (300×.)

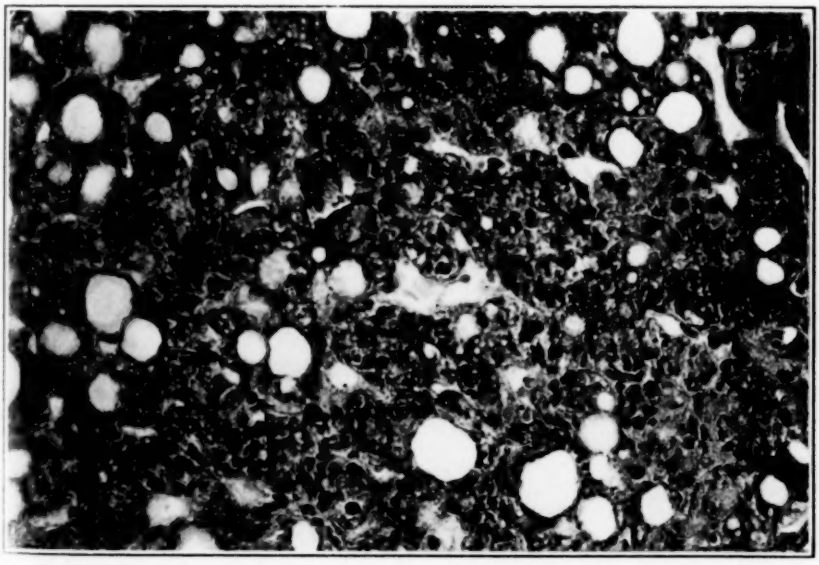

FIG. 2.—A section of the liver with cell degeneration prominent. There are intracellular fat vacuoles of varying sizes, pigment retention, swelling and granular degeneration of the liver cells and an infiltration with leukocytes. (400×.)

On May 3d, just before the splenectomy, samples of the patient's serum and cells were obtained for a more detailed study* of the serologic characteristics of this hemolysin. The reagents were prepared as follows: Erythrocytes: specimens of Groups AB, A, B and O cells and the patient's cells were washed three times with physiologic saline solution; suspensions containing 2% of cells were prepared; three different normal donors of each blood group were employed. Serum: one portion of the patient's serum was inactivated at 55° C. for 30 minutes; the other portion was used without heating; the patient's serum and cells had stood in the ice box for 48 hours before the first tests were carried out. Complement: a 1:10 dilution of fresh guinea pig serum in physiologic saline solution was used (the number of units of complement was not determined); fresh human sera of appropriate blood groups was also used with similar results. The procedure was carried out as follows: after mixing the reagents (red cell suspension 0.2 cc., serum 0.4 cc., complement 0.2 cc., saline 0.2 cc.) the tubes were incubated for 1 hour at 37° C.; after a preliminary reading, incubation was continued in the ice box over night.

Observations. 1. Uninactivated patient's serum, with or without added complement, showed marked hemolysis of normal Group A, B and O cells, slight hemolysis of Group AB cells, but no hemolysis of the patient's cells. 2, Inactivated patient's serum, without the addition of complement, showed no hemolytic action. 3, Inactivated patient's serm plus complement hemolyzed the patient's cells and the normal Groups AB and A cells in dilutions up to 1:16 but not in 1:24. 4, After standing in the ice box for 3 weeks the hemolytic titer of this preoperative serum decreased to a dilution of 1:8 both for the patient's postoperative cells and for fresh specimens of normal Groups AB and A cells. 5, When this 3 weeks' old serum was mixed with normal Group B serum and allowed to stand for 10 minutes at room temperature before the patient's postoperative cells and complement were added, hemolysis did not occur (in the 1:4 dilution of serum) during the usual incubation. 6, The patient's 3 weeks post-splenectomy, fresh or inactivated serum, without the addition of complement, did not hemolyze the patient's postoperative cells or the normal cells of Groups A, B or O; if complement was added, hemolysis of Group A cells occurred in a dilution of 1:16; normal Group AB cells were not tested. 7, The ice box incubation did not increase the degree of hemolysis over the amount present at the end of the 1 hour incubation at 37° C. in any of these tests. 8, Preliminary incubation in the ice box or at room temperature, before the 1 hour incubation at body temperature, did not alter the hemolytic action in any of these tests.

In summary, the hemolysin that was active against the patient's cells (*a*) required complement, (*b*) did not require cooling, (*c*) decreased in activity on standing in the ice box for 3 weeks, (*d*) was neutralized by normal homologous serum and (*e*) was active against the patient's 3 weeks post-splenectomy cells as well as the preoperative cells. This hemolysin was not present in the patient's serum 3 weeks after the splenectomy.

An uninactivated, sterile saline extract of minced splenic tissue incubated in the same manner with and without the addition of complement was actively hemolytic for normal cells of Groups A, B and O, slightly hemolytic for normal Group AB cells and not at all for the patient's B cells. This is identical with the peculiar hemolytic property of the patient's uninactivated preoperative serum that was removed by heating. Heat inactivation of the splenic extract resulted in the formation of a heavy precipitate; no hemolytic action was demonstrable in the supernatant fluid. Aërobic and anaërobic cultures of this spleen, made at the operating table, were sterile.

* Conducted by E. H. Spaulding, Ph.D., of our Department of Bacteriology.

Discussion. The clinical condition of the patient, the biochemical evidences of impaired liver function and the histopathology of the liver indicate that this patient's severe crisis was due to an acute liver failure. The clinical evidences of impaired liver function were: anorexia succeeded by nausea and vomiting, vague epigastric discomfort, delirium, coma, fever, tachycardia, hypotension, increasing hepatomegaly and marked icterus. The laboratory evidences (Table 1) of liver dysfunction were: a decrease in the esterified portion[11] of the total serum cholesterol; an increase in the urinary urobilinogen excretion;[25b] the change of the van den Bergh reaction (Chart 1) from indirect to direct[22] without evidence of obstruction of the extra-hepatic biliary tract; the appearance of bile pigment in the urine at the height of the crisis; a decrease in the total blood serum protein concentration;[12] and the cholesterin crystals and black pigment in the biliary drainage material.

The rapid clinical and laboratory recovery of liver function in the face of such severe liver damage (Fig. 2) suggests that the spleen was responsible for the degeneration of the liver. The extensive fatty and pigmentary degeneration with peri-portal infiltration of neutrophils revealed in the liver biopsy does not appear in other types of severe anemia. Similar lesions have been reported at the autopsy of patients with hemolytic anemia.[7,8,19] Pigmentary degeneration of the liver with fatty degeneration of the parenchymal cells in the central portion of the liver lobule has been recognized in the chronic hemolytic anemias.[1,20,22] Fatal transfusion reactions produce scarcely any histologic liver damage in patients without preëxisting hemolytic anemia.[9] The frequency of severe clinical crises and the greater severity of the anemia characterize the acquired form of hemolytic icterus in contrast with the familial variety.[17] The observations on this patient indicate that an acute liver failure is one pathogenic mechanism of the clinical crises characteristic of the hemolytic anemias.

The rise of the red blood cell count from 780,000 to 2.6 million during the first 18 hours after splenectomy indicates that the spleen was responsible for the previously falling erythrocyte count and the increasing serum bilirubin concentration. In spite of the marked anemia prior to splenectomy, the small number of normoblasts and reticulocytes suggests that some factor inhibiting erythropoiesis,[2,10] as well as a hemolytic factor, was removed by the splenectomy. The marked normoblastic response and the transient neutropenia and thrombocytopenia following the operation likewise suggest that red blood cell formation had been depressed by some action of the spleen.

Pepper's[21] classification of the hemolytic anemias according to the mechanism of hemolysis may be rephrased as follows:

A. Familial. 1, Congenital hemolytic jaundice (spherocytosis); 2, sickle cell anemia.

B. Acquired. 1, Allergic (favism, drugs, etc.); 2, chemical (industrial exposure, drugs, etc.); 3, infectious (Strep. hemolyticus, bacillus Welchii, ? Lederer's acute hemolytic anemia, etc.); 4, infestations (malarial plasmodium, etc.); 5, physical (cold in paroxysmal hemoglobinuria); 6, symptomatic (leukemia, Hodgkin's disease, tuberculosis, etc.); 7, idiopathic (such as paroxysmal nocturnal hemoglobinuria); 8, the hemolysinic anemia, first described by Chauffard and Vincent,[4] may be added to this classification.

This case of macrocytic, hemolytic anemia may be classified as a *hemolysinic* anemia. Although there was chronic icterus and previous severe episodes of jaundice, there was no family history of jaundice and the red blood cells have shown neither increased fragility in hypotonic saline solution nor spherocytosis during 3 months of close observation. A hemolysin was present in the blood serum prior to splenectomy and the case resembles Cases 1 and 2 reported by Dameshek and Schwartz;[6a] the hemolysin is likewise similar.[6b] Krumbhaar[17] and Dameshek[5] have reviewed the previously reported cases of this type. The clinical response to splenectomy, the histopathology of the spleen and the disappearance of the hemolysin from the blood serum after splenectomy suggest that the spleen was responsible for the serum hemolysin, the hemolytic anemia and the liver damage.

In the hemolytic anemias, the primary diagnostic efforts should be directed toward a determination of the mechanism of hemolysis,[21] for in many of these types of hemolysis splenectomy is either not indicated or has proved of little or no value.[24] Having excluded the known causes of hemolysis and demonstrated a hemolysin in the blood serum, splenectomy is indicated to remove the organ which is causing the hemolysis and injury to the liver. In Lederer's acute hemolytic anemia, which is usually a macrocytic anemia, transfusions are adequate therapy.[15,18] However, transfusions must be used with caution in the hemolytic anemias because of the danger of aggravating the hemolysis. This danger has been emphasized by Sharpe and Davis[23] and is illustrated in this and many other cases.[6a, 8, 10, 13]

To clarify the problem of ictero-anemia, blood serum hemolysins should be searched for in all types of hemolytic anemia until adequate information on the nature and rôle of these antibodies is available. Dameshek and Schwartz[6b] believe that spherocytosis represents a milder rate or degree of the action of a serum hemolysin than the macrocytic type of hemolytic anemia such as was present in this case and in 2 of their cases.[6] Furthermore, Hill[13] has reported

that microspherocytosis was present only during some stages of the course of clinically typical cases of congenital hemolytic jaundice.

Conclusions. A case of *hemolysinic* anemia showed a macrocytic type of anemia in its third critical episode during 2 years' time. Transfusions aggravated the anemia; the symptoms and laboratory evidences of severe liver dysfunction became apparent.

A hemolysin was demonstrable in the blood serum before but not after the splenectomy.

Splenectomy was performed successfully on a moribund patient as an emergency measure in the treatment of the hemolytic anemia and the acute liver failure.

The clinical, hematologic and biochemical response to splenectomy was astonishingly complete and rapid although the patient was shown by biopsy at the time of operation to have severe fatty and pigmentary degeneration of the liver.

[Hemolytic anemia, in the broad sense of the term, can have many causes and mechanisms. For instance, congenital hemolytic jaundice (of which the so-called acquired form is now regarded as merely the activation of a latent stage) appears to be due to a congenital defect of the erythrocyte (spherocytosis) that makes it more susceptible to rupture by osmotic change. In the present case, which obviously is not one of congenital hemolytic jaundice, a hemolysin was clearly demonstrated and the dramatically successful results of splenectomy showed either that the hemolysin was formed in the spleen or at least that the presence of the spleen was a requisite for the production of liver damage. Such cases are rare. It is possible, however, that careful serologic studies will show hemolysins in a greater proportion of cases. Some years ago, studying artificial plethora, we found that with repeated injections of blood, polycythemia was eventually replaced by anemia of increasing severity; and yet we were not able to demonstrate the hemolysins that we felt were probably present. Perhaps some of the clinical hemolytic anemias fall in a similar category. I am glad to comply with the authors' request to add this supplementary note.—ED.]

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ERYTHROCYTE MORPHOLOGY IN EXPERIMENTAL HEMOLYTIC ANEMIA AS INDUCED BY SPECIFIC HEMOLYSIN.*

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Because of the interest aroused by the recent report of Dameshek and Schwartz[4] suggesting the relationship of human hemolytic anemias and the experimental hemolytic anemias produced in animals by injection of species-specific hemolytic serum, and because the data so obtained are comparable to certain types of blood pictures seen clinically, the results of this preliminary investigation of the subject are reported.

The study was undertaken in an attempt to establish a "standard" hematologic reaction in the dog following a single injection of anti-dog cell hemolysin. The findings will be utilized in future study as a basis of comparison or contrast in animals receiving hemolysin after some other alteration in erythrocyte dimensions or metabolism.

Method. *Preparation of Hemolysin.* Rabbits were given 5 intravenous injections of 1 cc. of 20% suspension of thrice washed dog red cells in normal saline at 3- or 4-day intervals. The animals were bled 7 days after the last injection.

Titration. To 0.5 cc. of progressive dilutions of the rabbit serum were added 0.5 cc. of a 2% suspension of washed dog cells, 1.5 cc. of 0.85% saline and 0.5 cc. of a 1 to 10 dilution of fresh guinea-pig serum. This was followed by incubation for 1 hour at 37° C. Lysin titers are expressed in terms of the final highest dilution at which definite hemolysis was visible. Titers as high as 1 to 9600 were obtained. Agglutinin titers, expressed in the same terms, were equal to or slightly higher than the respective hemolysin concentrations.

After inactivation at 56° C. for 30 minutes the sera were preserved undiluted by freezing.

Production of Anemia. Eleven mongrel dogs, on a diet of commercial dog biscuits, were given single intraperitoneal injections of varying amounts of hemolytic serum.

Hematologic Methods. Determinations were performed daily at the height of the anemia and at longer intervals thereafter. Blood was obtained from the ear vein except for hematocrit determinations. Blood was drawn from the cubital vein in a syringe coated with mineral oil for this determination. Heparin (Connaught) was used as an anticoagulant in the concentra-

* This investigation was aided in part by a grant from the Fraser Fund, Baylor University College of Medicine.

tion of 2 units per cubic centimeter of blood. In 2 of the animals all determinations were carried out with heparinized blood.

Bureau of Standards red cell pipettes and counting chambers were used throughout, counting at least 10 squares after mechanical shaking of the pipettes. Hayem's solution was used as a diluent, frequently being checked with Gower's solution[7] if clumping of the red cells was marked. The majority of the counts were performed by one individual. Hemoglobin estimations were made by the Newcomer method. Wintrobe's hematocrit tubes[15] were used and were read after centrifugation for 1 hour at 3800 r.p.m.

Reticulocyte counts were made by the damp-chamber method using brilliant cresyl blue and counterstaining with Giemsa. Cell diameters were obtained by projection and direct measurement at a magnification of 3000 diameters. The slides used were stained with Leishman's and were counterstained with 1% aqueous eosin. At least 250 cells were counted for each determination and in some instances this was increased to 500. The average diameter and the standard deviation were calculated by the method of Price-Jones.[12b] Red cell thickness was estimated by the formula:

$$\text{mean corp. thickness} = \frac{\text{mean corp. vol.}}{3.14 \text{ rad.}_2}.$$

Fragility tests were carried out according to the technique of Daland and Worthley.[3] Sparkman's method[13] was used for the urobilinogen determinations.

Results. *Normal Values.* The erythrocyte level of the animals used varied between 6.6 and 8 million cells per c.mm. (In 1, the initial blood count was 5.5 million.) Average hemoglobin values lay between 12.9 and 15.2 gm. The reticulocyte count was less than 1.5% in all instances.

The average cell diameter varied between 6.99 and 7.34 microns, with an average mean corpuscular volume of 68 ± 5 cu. microns. The standard deviation of the diameter, counting 250 cells, was less than 0.5 micron. The calculated cell thickness averaged 1.85 microns.

Hemolysis usually began around 0.44% saline and was complete at 0.32%.

Control Experiments. The injection of amounts of normal rabbit serum comparable to the amount of hemolytic sera used was followed by no significant change in cell size and only a slight reticulocyte response.

General Effects. Following the injection of the hemolysin, certain changes were observed in all of the animals, varying in intensity with the amount and titer of the hemolysin. The earliest change that could be detected without cell measurements was a decreased resistance of the red cells to hemolysis by hypotonic saline. This was present after 4 to 12 hours, becoming more evident as cell destruction began and hemoglobinemia developed. With the development of hemoglobinemia the level of the red cells and hemoglobin began to decrease, usually reaching the lowest reading in 3 to 6 days. With larger amounts of hemolysin destruction of nearly all of the circulating erythrocytes was observed, with a cor-

responding lesser destruction with smaller amounts of lower titers. Examples of the levels of anemia produced are shown in Chart 1.

If the amount of hemolysin administered was large, death resulted at this stage. If smaller doses were given, the animal usually maintained the red cell level reached for 2 to 4 days, following which there was a gradual return of the red cell count and hemoglobin toward normal readings.

Prior to the rise in red cell count examination of the stained blood films showed definite signs of bone-marrow response. If the demand were only moderate, an increase in the number of reticulocytes was the only change. If the demand were more severe, nucleated red cell forms were frequent. Occasional megaloblasts were observed

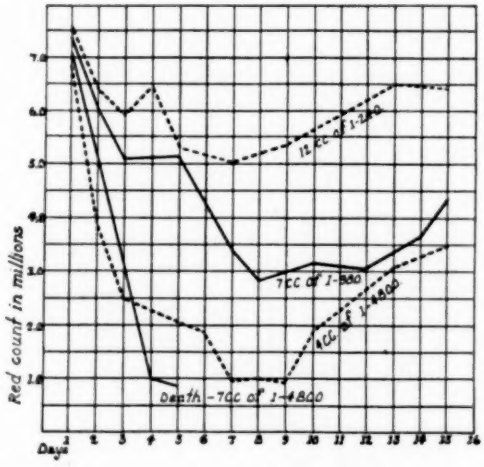

CHART 1.—The duration and severity of the depression in the erythrocyte level caused by a single injection of hemolysin. Each line represents a different animal.

in the most severe anemias, and a loss of phase between the nucleus and the cytoplasm of the erythroblasts and normoblasts was a common observation. The reticulocyte level occasionally reached 50% and numerous polychromatophilic cells were present in the recovery phase.

Pigment Excretion. With the exception of 1 animal, the hemoglobinemia was of sufficient degree to be accompanied by moderate or severe hemoglobinuria. The hemoglobinuria, once established, continued for several days, usually diminishing in amount and disappearing after the red cell count started to rise.

Stool urobilinogen excretion showed marked increases as the anemia progressed, with the highest readings obtained about 24 hours after the low level of the red cell count was reached. In

some instances the output was as high as 900 mg. per 100 gm. of stool (approximately 30 times the normal excretion).

Hematocrit Readings. During the first 3 to 5 days the volume of packed cells decreased in proportion to the drop in erythrocytes. At this stage, in some instances, hematocrit readings were inaccurate because of the varying amounts of cell débris present. Occasionally, on the third or fourth day a slight rise (3 to 4%) was noted, which was not associated with demonstrable cell débris. During the recovery phase a definite rise in the hematocrit levels as compared to the rise in red cell count was present, the amount varying directly with the immaturity of the cells. Mean corpuscular volumes of 126 and 123 cu. microns were the extremes recorded.

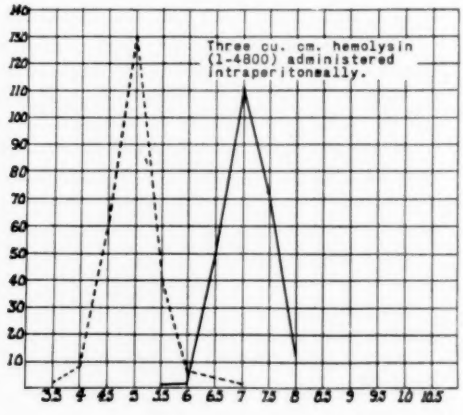

CHART 2.—Price-Jones curves illustrating extreme changes in cell diameter following a single injection of hemolysin. The solid line shows the normal curve; the dotted line, the curve obtained 2 days later. The standard deviation of both curves is less than 0.5.

Erythrocyte Diameters. Following the injection of a large amount of hemolysin there was observed a marked decrease in the diameter of the erythrocytes. This change was demonstrable in 12 hours, but usually reached the low level in about 48 hours. In 1 instance there was a drop from 7.2 to 4.9 microns (Chart 2), with proportionately smaller diminutions in diameter following smaller amounts or lower titers of injected hemolysin.

The standard deviation of this type of cell population closely approximated the normal standard deviation, or in some instances, was less than the normal standard deviation.

Usually on the third or fourth day there was observed a significant increase in the standard deviation associated with a widening of the base of the curve, due to the appearance of cells of normal size or larger. Within 2 or 3 more days the presence of two separate

cell components could be demonstrated, as shown by the bi-modal curves obtained (*i. e.*, two peaks in curve of cell diameters). It is significant to observe that in some instances the modes of the curves (as those shown in Chart 3, eighth day, and Chart 5, eleventh day) were separated by a distance greater than 3 times the standard deviation of the component of cells with decreased diameters.

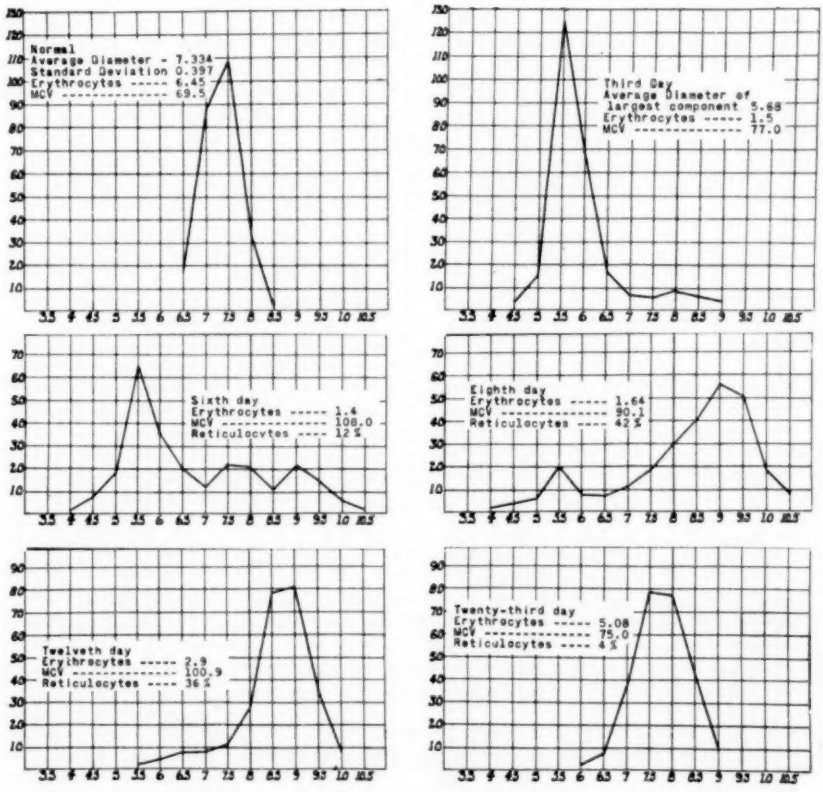

CHART 3.—Price-Jones curves illustrating the decrease in diameter of the circulating erythrocytes after a single injection of hemolysin, followed by gradual diminution in the number of these cells and their almost complete disappearance by the twelfth day. Note that the cells from the bone marrow are at first relatively normal in size but soon shift markedly toward larger cells following which the entire new cell population gradually shifts back toward normal size. Statement of the mean cell diameter in certain of these curves would give an entirely erroneous notion as to the type of cells present.

The further course of these bi-modal curves is of much interest. The cells of small diameter gradually decreased in number, occasionally seeming also to decrease in diameter (Chart 5), persisting in the blood stream for periods up to 1 month following injection

of the hemolysin (Chart 4). The length of survival of these cells was inversely proportional to the amount of hemolysin administered.

The other component of the curves, after small doses, consisted of cells of normal diameter or of only slightly increased diameter (Chart 4). With larger amounts of hemolysin the curve first was

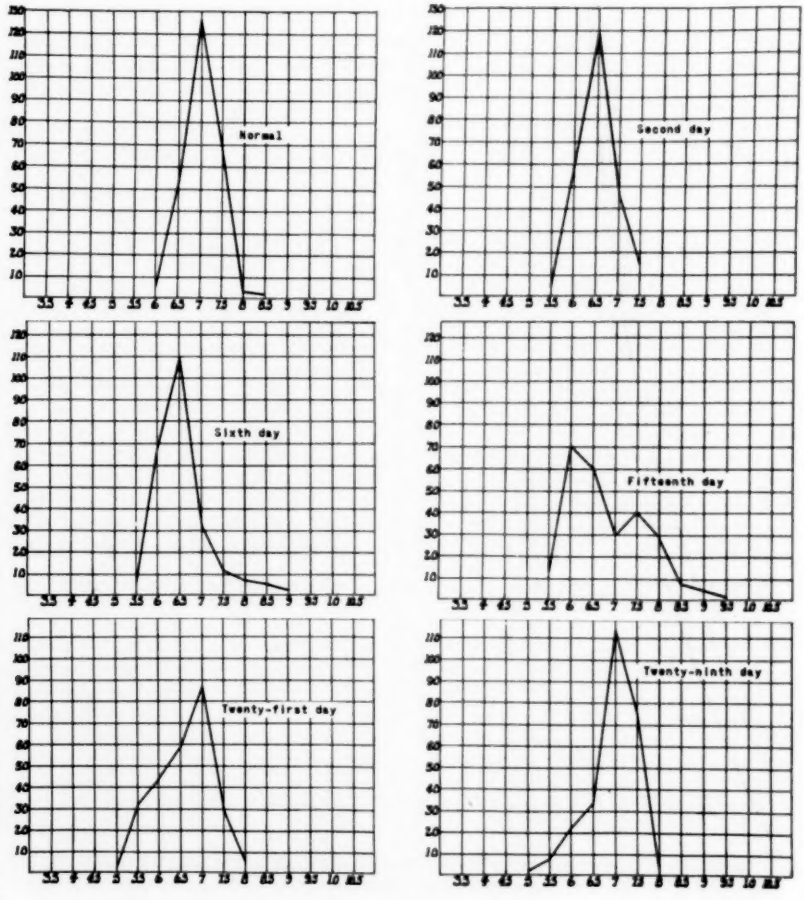

CHART 4—Price-Jones curves illustrating a slight decrease in diameter of the circulating cells with a response of nearly normal sized cells. Note that the left component persists in all of the curves shown and that the smaller cells in this component show a relative increase in number.

similar but soon shifted so that cells with large diameters formed its main component. As the blood count rose the group of large cells increased in number, and at the same time the average diameter tended to shift back toward normal figures, usually approaching normal in 3 to 4 weeks. In some instances, particularly following

small amounts of the hemolytic serum, there was only a slight decrease in the diameter of the circulating cells and the response was with cells of nearly normal diameter, so that the two components were more or less combined, forming a uni-modal curve with a broad base and a high standard deviation (Chart 4, twenty-first day).

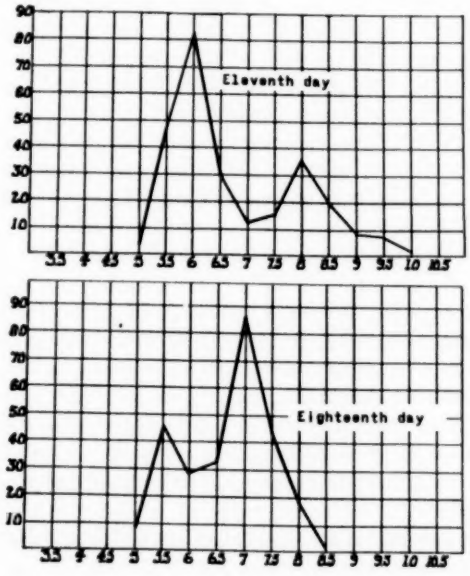

CHART 5.—Bi-modal Price-Jones curves showing the slightly macrocytic reponse after injection of hemolysin and the progressive decrease in the diameter of both components. The cell diameter prior to injection was 7.12 microns. Note that cells of decreased diameter persist even when the right component is normal in size.

Examination of the stained blood film and of wet preparations gave the impression that the small cells were definitely thicker than normal. Correlation of the diameter and the mean corpuscular volume indicated that the mean corpuscular thickness was definitely increased over the normal figure. The table below contains examples of the figures obtained on the second day.

TABLE 1.—CHANGES IN SIZE AND SHAPE OF ERYTHROCYTES DURING HEMOLYSIS.

Animal No.		Mean corp. vol. (cu. microns).	Diameter (microns).	Thickness (microns).
2	Normal	77.7	7.12	2.0
	Second day	77.8	6.4	2.35
4	Normal	69.5	7.3	1.65
	Second day	71.8	5.7	2.8
7	Normal	65.9	7.1	1.7
	Second day	71.0	5.5	3.0

As the blood smears were followed the large cells which form the second component were observed to exhibit either definite polychromatophilia or reticulation, or to belong to the nucleated group.

On the fifth or sixth day after very large doses of hemolysin, a rare microcyte may be seen which contains only one or two strands of poorly staining reticulum. With this exception, the young cells were not observed to show the diminutions in diameter that was present in the mature forms.

Fragility. The change in the fragility of the red cells following the injection of the hemolysin seemed to be roughly proportional to the diminution in diameter and to the corresponding change in the cell thickness. In some instances the cells were so fragile, or hemolysis was proceeding at such a rapid rate, that it was not possible to obtain a hemoglobin-free supernatant fluid even after multiple washings with normal saline. This was particularly true in the acute severe anemias, and in 1 instance all of the cells were hemolyzed in 0.8% saline.

An increased fragility was present as long as the component of cells with small diameters could be demonstrated. However, with the appearance of the larger cells the level of complete hemolysis returned to normal levels, giving a lengthened range of hemolysis. Occasionally, at this stage, hemolysis was not complete until 0.28% or 0.26% saline was reached.

Comment. The production of anemia by the injection of hemolytic serum is an old procedure. For a survey of previous observations, reference should be made to the report of Dameshek and Schwartz.[4]

The present findings essentially confirm those previously reported. In normal animals the decrease in diameter of the individual circulating erythrocytes was associated with little or no change in cell volume. This can only be interpreted as meaning that the cell is increasing in thickness or is approaching "sphericity" with the associated increase in fragility. This type of change toward "sphericity" is in sharp contrast with the definite increase in cell volume (associated with slight change in diameter) observed in hypotonic hemolysis by Haden[8] and by Castle and Daland.[2] Stewart[14] observed in 1899 that in hemolysis under the influence of serums, there was no marked increase in the electrical conductivity, but hemolysis by water caused an increase of conductivity, offering evidence that the mechanism of hemolysis is different with different agents.

After 3 to 6 days a definite increase in the mean cell volume is evident. This is associated with an increasing reticulocyte count or an inpouring of even younger cells into the blood stream and compares with the increase in the number of cells with normal or increased diameters observed in the Price Jones curves of these respective days.

Price-Jones curves with widely separated and distinct modes are rarely reported in the literature. Although the transfused red cells in patients with severe pernicious anemia may be recognized by

their normal contour, as contrasted with the bizarre cells present in this condition,[1] they do not cause a bi-modal curve because of the large standard deviation present. A study of the same type in a familial hemolytic anemia with an average cell diameter of 6.6 microns showed a definitely bi-modal curve following transfusion.

Bi-modal curves having shallow depression are presented by Momigliano-Levy and Bairati[10] in cases of familial hemolytic anemia following splenectomy. Also, Dameshek and Schwartz[4] illustrate a bi-modal curve with small macrocytic component during the recovery phase of experimental anemia in guinea-pigs, stating that later the curve may show a more definite macrocytic tendency.

Price-Jones[12b] and Mogensen[9] discuss the possibility of bi-modal curves occurring, and the latter author presents a method for the decomposition of atypical curves into their component parts. This method is somewhat complicated and unnecessary for the curves here presented.

Examination of curves obtained in this series shows that those cells altered in diameter do not all undergo immediate hemolysis. These altered cells remain in the blood stream for varying periods, dependent upon the amount of hemolysin administered. Following small amounts of hemolytic serum, with less change in diameter, they may persist in detectable numbers for approximately 1 month. This particular phenomena suggests an explanation for the failure to demonstrate hemolysin in the serum of many human hemolytic anemias, since, in some instances, mature erythrocytes of normal dimensions may be present in the blood stream with these spherical cells. Serologic investigations, to be reported at a later date, suggest that (with certain exceptions) demonstration of the hemolysin in the serum, even in experimental animals, may be difficult or impossible with the present methods. If correlation with *in vitro* activity of hemolysin is expected, demonstration of circulating hemolysin would rarely be possible unless some factor prevented its combination with erythrocytes.

These small cells, admittedly altered in certain of their characteristics, sufficient to permit identification, persist in the blood stream for a period fully as long as that previously reported for duration of life of the mature erythrocyte of this animal. Eaton and Damren[5] give 16 days based on periods of regeneration while Escobar and Baldwin[6] place the duration at 16 to 23 days, using the response to change in oxygen pressure.

The other component of the curves may be composed either of cells of nearly normal diameters or of cells of increased diameter. A macrocytic response was obtained in this same type of experimental anemia by Dameshek and Schwartz[4] and by Muir and McNee.[11] Price-Jones[12a] shows a similar response after phenylhydrazine poisoning in rabbits, but the data do not permit the plotting of curves.

The course of these curves suggests maturation of these cells in the blood stream, in that as recovery from the anemia progresses the average diameter tends to return gradually toward normal. This return is associated with a diminution in the reticulocyte count suggesting that the demand on the bone marrow is lessened and is associated with a normal or lowered pigment excretion.

When the two components of the curves are almost fused, rather than being widely separated, the picture closely simulates certain phases of human hemolytic anemias, particularly "atypical" cases.

The findings presented suggest an explanation for the cell destruction, "spherocytosis," increased fragility, and certain other phases of human hemolytic anemias. They do not afford an explanation for some features seen in certain of these cases. It is possible that these changes may be obtained with multiple injections of hemolysin on cells previously altered in size and shape. Further work is now in progress along these lines.

Conclusion. 1. The administration of a specific hemolysin to the donor animal (dog) is followed by a fall in the erythrocyte and hemoglobin levels, proportional to the amount of hemolysin administered and to the titer of the hemolysin.

2. In the process of cell destruction due to hemolysin (in contrast to the mechanism of hypotonic hemolysis) the erythrocytes approach a spherical form by a diminution in diameter associated with little or no increase in corpuscular volume.

3. This approach to sphericity is paralleled by proportionately decreased resistance to hypotonic hemolysis.

4. The length of life in the blood stream of the erythrocytes altered by the hemolysin is inversely proportional to the amount and titer of the hemolysin administered, and survival periods up to 1 month have been observed.

5. Factors pointing toward maturation of the cells entering the blood stream as response to the anemia are discussed.

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THE EFFECTS OF SULFANILAMIDE AND SULFAPYRIDINE UPON THE BLOOD PIGMENTS OF WHITE RATS.

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In a recent paper in this journal Machella and Higgins[3] report that the daily administration to white rats of sulfanilamide, 1000 mg. per kg., produced a marked cyanosis; whereas Wendel[7] states that in his experience sulfanilamide did not produce methemoglobin in rats or in dogs, rabbits and mice. It was then of interest to determine whether the cyanosis observed in rats was due to some derivative of sulfanilamide as has been suggested[5] or to some other altered hemoglobin such as sulfhemoglobin. Machella and Higgins[3] found that the cyanosis from neoprontosil and sulfapyridine at the same dose level was less pronounced.

In the present study we have determined the concentrations of methemoglobin, sulfhemoglobin and total hemoglobin in the blood of rats that receive daily doses of sulfanilamide* or of sulfapyridine.*

Procedure. Adult white rats were for 30 days given daily doses by mouth of a 2% acacia suspension of the drug. The concentration of the drug was so adjusted that they received 10 cc. of the suspension per kg. body weight. The daily dose of sulfanilamide was 700 mg. per kg. or approximately one-tenth the 50% fatal dose as determined by Molitor and Robinson.[4] Since the sulfanilamide portion of the sulfapyridine molecule is probably the part associated with methemoglobin formation it was decided that, for purposes of comparison, approximately equi-molecular doses of the two should be given. The dose of sulfapyridine was, accordingly, 1050 mg. per kg.

Methemoglobin, sulfhemoglobin and total hemoglobin were determined at the end of the 30-day period by the method of Evelyn and Malloy,[2] using the Evelyn photoelectric colorimeter. By this method each of the pigments is probably determined with an error of less than 0.2 gm. per 100 cc. Blood was obtained by clipping the tail.

Results. The results are summarized in Table 1.

TABLE 1.

Control.			Sulfanilamide.			Sulfapyridine.		
M.	S.	T.	M.	S.	T.	M.	S.	T.
0.2	0.2	13.1	0.5	1.6	13.9	0.2	0.2	14.1
0.2	0.2	14.8	1.0	1.4	13.0	0.4	0.3	14.8
0.4	0.1	14.0	0.6	2.0	13.3	0.4	0.7	14.4
0.3	0.2	14.3	0.6	2.2	13.3	0.7	0.2	15.9
0.2	0.1	13.2	0.5	1.2	13.1	0.7	0.1	15.4
0.2	0.1	13.9	0.5	0.8	12.1	0.7	0.1	13.8
0.2	0.1	14.2	0.8	1.4	13.2	0.7	0.2	12.4
0.2	0.2	16.5	0.8	1.3	13.6	0.7	0.2	13.4
0.3	0.1	16.1						
Mean:								
0.2	0.1	14.5	0.7	1.5	13.2	0.6	0.2	14.5

M = Methemoglobin in grams per 100 cc.
S = Sulfhemoglobin in grams per 100 cc.
T = Total hemoglobin in grams per 100 cc.

* The sulfanilamide was kindly furnished by the Winthrop Chemical Company; the sulfapyridine, by Merck & Co.

The small amounts of methemoglobin and sulfhemoglobin in the blood of the control animals is frequently greater than the error of the method and is in accord with the report of Ammundsen[1] who found small, but significant, amounts of non-oxygen-combining pigments in the blood of normal subjects. The amounts of methemoglobin formed after sulfanilamide and sulfapyridine were similar but not large. Whereas there was almost no sulfhemoglobin in the blood of rats receiving sulfapyridine there was twice as much sulfhemoglobin as methemoglobin in rats receiving sulfanilamide. In no case was the total altered pigment more than 3 gm. per 100 cc. This was correlated with the observation that none of the animals showed a marked cyanosis. Only in the case of sulfanilamide was there a significant reduction in the total hemoglobin. This is in general agreement with the results of Richardson[6] and of Machella and Higgins[3] who found greater blood destruction after sulfanilamide than after sulfapyridine.

Summary. 1. In white rats that had received orally for 1 month daily doses of sulfanilamide, 700 mg. per kg., small, but significant, amounts of methemoglobin and somewhat larger amounts of sulfhemoglobin were found. By comparison with a group of control rats there was a decrease in total hemoglobin of about 10%.

2. Under similar conditions a daily dose of sulfapyridine, 1050 mg. per kg. resulted in a similar amount of methemoglobin without an appreciable amount of sulfhemoglobin. There was no significant reduction in total hemoglobin.

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CORONARY EMBOLISM: A COMPLICATION OF SYPHILITIC AORTITIS.

With Report of 3 Cases.

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Occlusion of coronary arteries by thrombosis is a relatively common occurrence, but occlusion by an embolus is rare. In reviewing the autopsy records at the Medical College of Virginia, 3 cases were found in over 3000 autopsies. These cases are of

special interest because the occlusion in each case is the indirect result of syphilitic aortitis. The electrocardiographic tracing in the third case is apparently the only one ever to be recorded in a case of coronary occlusion by an embolus.

Case Reports. CASE 1.—H. C., a colored male, aged 37, was awakened suddenly at 10 P.M. on Dec. 16, 1936, with a violent pain in his chest and mid-epigastrium; he became nauseated and began to vomit. These symptoms continued and got worse; at 2 A.M. he was brought to the St. Philip Hospital emergency room. His past history revealed that he had had several attacks of "acute indigestion," but other than this there was nothing of significance. He was apparently in good health when he retired on December 16.

Examination revealed a well-developed and -nourished colored male, acutely ill, perspiring freely, thrashing about in bed, vomiting copious amounts of undigested food, coughing, and complaining of severe pain in his chest and mid-epigastrium. His temperature was 96°, neither radial pulse could be felt, and his blood pressure did not register. There was marked distention of his neck veins. The heart sounds could not be heard. The chest was normal to percussion and auscultation. The abdomen was relaxed, no masses were felt, and there was tenderness on pressure in the epigastrium. The extremities were cold and moist.

At 3 A.M. the patient became more restless, his blood pressure still did not register, and his pulse could not be felt. He died at 3.45 A.M.

Autopsy. Syphilitic aortitis, mural thrombus in the arch of the aorta, embolus in the orifice of the left coronary artery, acute dilatation of the heart. Pulmonary edema.

Gross Description (abstract of protocol). The heart is moderately enlarged and both ventricles are considerably dilated. The epicardium, endocardium and valvular apparatus are intact. The myocardium is slightly relaxed, but not soft, and shows no foci.

Coronary arteries: There is no sclerosis. The left coronary artery is occluded at its mouth by a cylindrical antemortem thrombus.

Aorta: The ascending aorta is normal in caliber and reveals the characteristic findings of syphilitic mesaortitis with slight narrowing of the mouth of the coronary artery. There is grossly little evidence of atherosclerosis. Immediately above the commissure between the left and right aortic cusp there is a mural thrombus firmly attached to the aortic wall.

Lungs: Both lungs are heavy, soggy, and sections show large amounts of clear fluid. There are no other lesions in the lungs.

Microscopic Description. Heart: There is no evidence of myocardial degeneration or necrosis.

Aorta: There is marked fibrosis and thickening of the intima with overlying attached thrombotic material composed chiefly of fibrin and platelets. The media reveals severe scarring, metallaxis and marked vascularization. There is dense lymphocytic and plasma cellular infiltration and disintegration of elastic fibers. The inflammatory infiltration about the vasa vasorum in the adventitia is even more extensive and severe than the lesions in the media.

CASE 2.—W. B., a 32-year-old colored male, was in good health until 9.30 A.M., Jan. 7, 1939. While delivering ice he was taken suddenly with a severe pain in his chest which caused him to fall to the floor. He remained conscious and was brought to the St. Philip Hospital emergency room. His past history revealed nothing of significance.

Examination showed a well-developed and -nourished colored male propped up in bed, with short rapid respirations, cold clammy skin, and suffering from severe substernal pain. His temperature was 98.6°; neither radial pulse could be felt; his blood pressure was 65/50. The heart sounds

TABLE 1.—CASES OF CORONARY EMBOLISM.

No.	Author.	Year.	Age.	Sex.	Source.	Branch.	Death.	Condition of C. A.	Condition of myocardium.
1	Virchow	1856	27	F	Endocarditis	Several	?		
2	Huber	1882	64	M	Thrombosis in l. cor.	L. D.	Sudden		
3	Koreczynski	1887	38	F	Endocarditis ?	L. D.	Sudden		
4	Hektoen	1892	32	M	Thrombus on aorta	L. M.	Sudden	Marked sclerosis	Fibrous myocarditis
5	Rolleston	1896	17	M	Thrombus on left ventricle	L. M.	Sudden	Few atheromatous plaques	No infarction
6	Oestreich	1896	32	M	Pedunculated thrombus on wall of aorta, closed orifice at right	L. M. and rt. orifice	Sudden	No sclerosis	
7	Chiari	1897	32	M	Thrombus on aorta	L. M.	Sudden		
8	Welch	1899	36	F	Endocarditis	L. M.	Sudden		
9	MacCallum	1905		M	Endocarditis	L. M.	Sudden		
10	Lamb	1913	35	M	Endocarditis	L. M.	Sudden		
11	Gallavardin	1913	63	M	Thrombus of ventricle	L. M.	Gradual		
12	Kaufmann	1922	35	M	Thrombus on aorta	L. M.	Sudden		
13	Murray	1926		M	Vegetation on aorta	?	Sudden	?	?
14	Wolff-White	1926	31	?	Endocarditis	L. M.	Sudden	?	?
15	Wolff-White	1926	43	F	Pelvic thrombosis	L. D.	Sudden	?	?
16	Wolff-White	1926	46	?	Endocarditis	L. D.	Sudden	?	?
17	Wolff-White	1926	23	?	Endocarditis	L. D.	Sudden	?	?
18	Thompson, Evans	1930	25	M	Teratoma of testis	Both	Sudden	?	?
19	Saphir	1933	35	M	Thrombus, rt. femoral vein	L. D.	Sudden	No sclerosis	No infarction
20	Saphir	1933	70	M	Thrombus in rt. coronary	R. D.	Gradual	Marked sclerosis	Infarction
21	Saphir	1933	70	M	Thrombus on aorta	R. M.	Sudden	Marked sclerosis, L. D. cor. art. occluded by old thrombus	Myocardial fibrosis
22	Jacobi, Kandler and Silverman	1934	47	F	Femoral thrombosis	L. D.	Sudden	No sclerosis	?
23	Hoseason	1935	36	M	Thrombus on ulcerated aortic cusp	R. M.	Sudden	?	Healthy
24	Garvin and Work	1939	32	M	Endocarditis	L. D. L. C. R. M.	Gradual	No sclerosis	Infarction
25	Garvin and Work	1939	44	M	Endocarditis	L. D.	Gradual	Few atheromatous plaques	Infarction—left vent.
26	Garvin and Work	1939	26	M	Endocarditis	L. D.	Sudden	Coronaries ?	Diffuse fibrosis—no infarction
27	Work	1939	45	M	Endocarditis	L. D. L. C.	Sudden	No sclerosis	No infarction
28	Porter and Vaughan	1940	37	M	Syph. aortitis	L. M.	5 hr. 45 min.	No sclerosis	No infarction
29	Porter and Vaughan	1940	32	M	Syph. aortitis	L. M.	1 hr.	Very slight atherosclerosis	No infarction
30	Porter and Vaughan	1940	24	M	Syph aortitis	L. D.	10½ hrs.	No sclerosis	No infarction

Key to abbreviations:
L. M. Main branch of left coronary artery (before bifurcation)
L. D. Descending branch of left coronary artery
L. E. Circumflex branch of left coronary artery
R. M. Main branch of right coronary artery (before bifurcation)
R. D. Descending branch of right coronary artery

were very distant. There was no arrhythmia, and no murmurs were heard. The abdomen and extremities were not remarkable.

The patient was given ⅓ gr. of morphine sulphate, 7½ gr. of caffeine citrate, and placed in an oxygen tent. Pulmonary edema developed and he died at 10.30 A.M.

Autopsy. Syphilitic aortitis with mural thrombi in the aorta, complete embolic occlusion of the left coronary artery, narrowing of the ostium of the right coronary artery, multiple myocardial scars, acute dilatation of the left heart, pulmonary edema.

Gross Description (abstract of protocol). Heart: Weight, 390 gm. Thickness of the ventricular walls: right, 0.3 cm.; left, 1.4 cm. The epicardium is smooth and glistening. The left ventricle is dilated, the apex rounded. The endocardium is smooth and glistening, and the valvular apparatus appears essentially normal.

Aorta and coronary artery: Immediately distal to the aortic valve there is a row of flat, elevated, smooth, porcelain-like plaques. The largest of these measures 1.5 cm. in diameter and encroaches upon the orifice of the left coronary artery. Rather firmly attached to it are several friable thrombi, pinkish gray in color, which resemble vegetations. Another plaque has involved the mouth of the right coronary artery and has caused considerable narrowing of its orifice. The inner surface of the beginning portion of the aorta in between the plaques shows marked longitudinal wrinkling and puckering and a discrete area of thinning of the aortic wall. The arch of the aorta shows several early atherosclerotic plaques. The descending portion of the aorta is practically free from arteriosclerosis. An embolism of reddish, friable material has completely blocked the first centimeter of the left coronary artery and protrudes from the orifice into the lumen of the aorta for a distance of 5 mm. The remaining portion of this coronary artery as well as the right coronary artery is entirely normal aside from very slight atherosclerosis.

Lungs: Weight—left, 1150 gm.; right, 1200 gm. The lungs are heavy and soggy, and on section, allow large quantities of clear fluid to escape. There are no other pathologic findings.

Microscopic Description. Heart: Numerous sections through the myocardium show many areas of myocardial scarring. There is, however, no evidence of acute necrosis.

Aorta: Sections through various portions of the aorta show markedly elevated atherosclerotic plaques of the intima with softening and calcification. The media reveals extensive destruction of its normal architecture by numerous scars, and foci of densely collected round cells composed of lymphocytes and plasma cells. These foci are irregularly distributed in patchy areas throughout the media and often found about new-formed vessels penetrating the media. There is also extensive perivascular round-cell infiltration in the adventitia. The media shows advanced scarring and disintegration of elastic fibers.

CASE 3.—W. W., a 24-year-old colored male, entered the St. Philip Hospital emergency room at 8.30 P.M., May 12, 1939, complaining of severe pain in the chest. One hour before admission, while sitting in a theater, he was seized with a severe epigastric and precardiac pain, became nauseated, left the theater and fainted. He was brought to the hospital by the city ambulance. He was conscious on arrival, he had no symptoms other than those mentioned, no similar previous attacks, and he had been in good health until the onset of his present illness.

Examination revealed a well-developed and nourished colored male, acutely ill, perspiring freely, very restless and suffering from severe pain in the chest. His temperature was 99°, pulse rate was 60 and extremely feeble, respiratory rate was 24, and his systolic blood pressure was 62 (diastolic unobtainable) in both arms. Head, eyes, nose, mouth, throat and glands were not remarkable. The neck veins were moderately dis-

tended. The chest was normal to percussion and auscultation. The heart sounds were distant and weak, no murmurs were heard, the rhythm was regular, the apex rate was 60. The apex impulse was neither seen nor felt. The abdomen was soft, but there was no tenderness, and no masses were felt. The extremities were not remarkable.

The patient was examined with the fluoroscope and a feebly pulsating heart observed. The pulsations of the left ventricle were extremely weak. The heart was aortic in type and slightly enlarged.

An electrocardiogram was taken and this showed an absence of the *P* wave in all four leads; an elevated *RT* segment in Leads 1 and 4; a depressed *ST* segment in Leads 2 and 3; and a deep *Q* wave in Lead 4. (Chart 1.) The ventricular rate was 75, and regular. This tracing suggests a nodal rhythm, damage to the intraventricular conduction system, and acute coronary occlusion.

The white blood count was 8850. The differential count was 71% polys, 25% lymphs and 4% monocytes. The patient was given $\frac{1}{4}$ gr. morphine sulphate at 9 P.M. and 10.30 P.M. and he received very little relief from either injection. At 1 A.M. he became dyspneic and cyanotic, his pulse was 76, irregular and weak; his blood pressure was 90/70. He was placed in an oxygen tent at 8 liters O_2 per minute. Morphine, $\frac{1}{4}$ gr. was given at 2.30 A.M. and 6.30 A.M.; at 7 o'clock he developed pulmonary edema and died a few minutes later.

Autopsy. Syphilitic aortitis with mural thrombus on the aortic wall, embolic occlusion of the left coronary artery at its orifice, acute dilatation of the left heart, pulmonary edema.

Gross Description (abstract of protocol). Thickness of ventricular walls: right, 0.3 cm.; left, 1.4 cm. The epicardium is smooth, moist and glistening. The left ventricle is markedly dilated with rounding of apex. There is, however, no flattening of the papillary muscles to be seen. The myocardium is flabby and mottled on the cut surface, streaked with numerous dark red petechial hemorrhages. The endocardium and valvular apparatus are intact.

Aorta: The wall of the aorta is somewhat inelastic. It shows at its beginning a wrinkling of its inner surface which cannot be stretched out. There are elevated pearly gray plaques at the site of all three commissures which are firm in consistency and which separate the cusps from each other. There is, however, no evidence of aortic insufficiency in that pseudovalves at the tract of outflow are missing, nor is the left ventricle hypertrophied, nor are the papillary muscles flattened as would be expected in chronic dilatation. Other than the described changes the aortic wall shows just a few atherosclerotic plaques in the arch and descending portion. At the commissure between the right and left aortic cusp the roughened surface of one of the above-mentioned plaques is covered with friable thrombotic material which is grayish red in color and adherent to the intima. This thrombus is elongated, measuring approximately 10 by 5 mm. at its base, protruding 1 to 2 mm. into the lumen.

Coronary arteries: The main branch of the left coronary artery reveals a compact reddish gray friable plug which is slightly adherent to its wall and which is lodged at its mouth, extending just into the circumflex and descending branch of the left coronary artery. It apparently completely obstructs its lumen. The remaining portions of the coronary arteries are elastic and reveal a smooth intima.

Lungs: Weight—left, 700 gm.; right, 800 gm. They show no other pathologic findings than extreme edema. They are soggy, and large quantities of clear fluid ooze from the cut surfaces.

Microscopic Description. Heart: Numerous sections through various portions of the myocardium do not reveal definite areas of necrosis. The only histopathologic findings are scattered neutrophils in some areas in the interstitial connective tissue with dilated capillaries engorged with red blood cells.

Leads

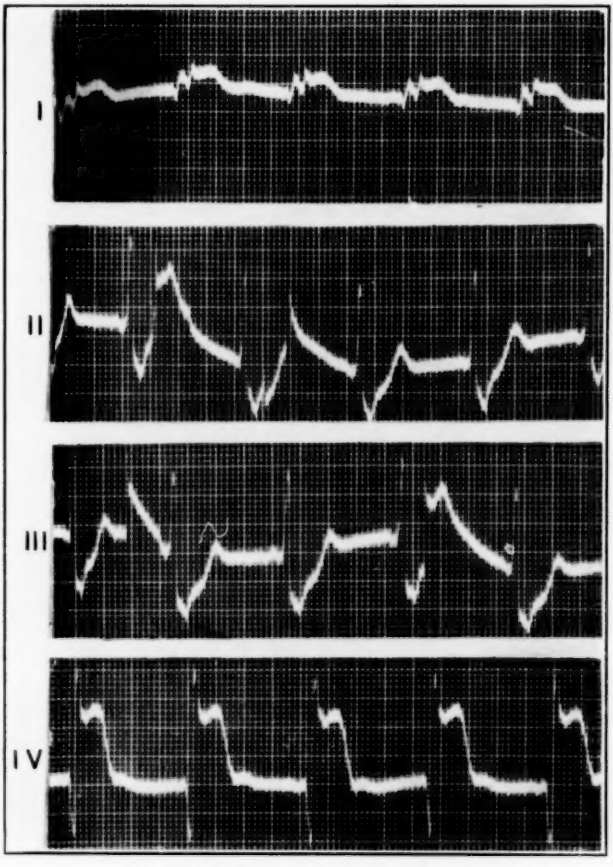

CHART 1.—Case 3. Electrocardiogram.

FIG. 1.—Case 3. Aorta showing atheromatous area with overlaying thrombus and syphilitic change in the media.

Aorta: Various sections through the beginning portion of the aorta reveal marked metallaxis of the latter with spotted areas of medial degeneration, "moth-eaten" disappearance of elastic fibers, vascularization of the media, marked perivascular round-cell infiltration with lymphocytes and plasma cells in the adventitia and media. One of the sections through the area overlying an atheroma reveals the thrombus to consist of fibrin, platelets and erythrocytes. The thrombus reveals evidence of early organization at its base with capillary budding. It is particularly noticeable that the atheromatous plaques of the intima are confined to such areas of the aortic wall in which there is marked destruction of the media by the syphilitic process (Fig. 1).

Coronary arteries: The thrombus in the lumen of the coronary artery is of the same type as that in the aorta, shows definite structure of an antemortem clot. The coronary arteries proper do not show histopathologic changes. There is no evidence of atherosclerosis. Organization of the clot has taken place.

Discussion. Saphir[7] reviewed the literature on coronary embolism from 1856 to 1933 and reported only 11 satisfactorily proven cases. Thirteen other cases were mentioned but either because of the brevity of the case reports or lack of autopsy findings, he did not believe they were satisfactorily proven. The 11 cases reported by Saphir will not be discussed here, but we wish to add to this list 7 cases, all mentioned by Saphir, because we believe they have been satisfactorily proven. In addition we shall add the 3 cases reported by Saphir and all other cases reported since 1933. For a case of coronary embolism to be satisfactorily proven, it must be proven at autopsy, and the source of the embolus should be clearly demonstrable.

Oestriech,[6] in 1896, cited the case of a 32-year-old male who died suddenly on his wedding night. Autopsy revealed an arteriosclerotic ulcer in the aorta covered by a thrombus; the thrombus occluded the mouth of the right coronary artery, and gave rise to an embolus which occluded the left main coronary artery. Murray,[5] in 1926, briefly mentioned the case of a young coal miner who died suddenly. Vegetations were found on the aorta and one of the coronary arteries was plugged by an embolus. He did not mention which coronary artery was occluded. Wolff and White,[9] in 1926, mentioned very briefly 4 cases of coronary embolism with sudden death. The left coronary artery was occluded in all 4 cases and the source of the embolus in 3 instances was an endocarditis. In the fourth case, the patient had a patent foramen ovale and the source of the embolus was a pelvic thrombosis. In 1930, Thompson and Evans[8] reported a case of sudden death in a 25-year-old male who had malignant teratoma of both testes. The autopsy findings were: a patent foramen ovale, a large polypoid mass of growth projecting from the left ventricle, and growth emboli in both coronary arteries. Saphir,[7] in 1933, described 3 cases, 2 of which died suddenly. In 2 cases, the right coronary artery was occluded; in 1, the left descending coronary. The sources of the emboli in the 3 cases were: femoral thrombosis, thrombus in the wall of the aorta, and a thrombus in the right coronary artery. In 2 of

these cases there was a marked sclerosis of the coronary vessels. Jacobi, Kendler and Silverman,[3] in 1934, described a case of sudden death in a 47-year-old female with femoral thrombosis. At autopsy a patent foramen ovale was found and an embolus was seen in the left descending coronary artery. There was no sclerosis of the coronary vessels. Hoseason,[2] in 1935, mentioned a case of sudden death in a 36-year-old male who became acutely ill with pain in the chest and epigastrium, nausea and vomiting. At autopsy there was ulceration of the aortic cusp with thrombus formation, and the right coronary artery was occluded by an embolus. Medlar,[4] in 1935, cited a case of coronary embolism with infarction of the myocardium. The patient had extensive bilateral pulmonary tuberculosis, and died rather suddenly. At autopsy, a recent infarction was found in the lateral and upper portion of the left ventricle, but on gross examination there was no evidence of thrombosis on embolism. In microscopic examination, in one of the small coronary arteries, caseous material containing tubercle bacilli was found. We do not feel that this case should be included in the list of satisfactorily proven cases because the embolus was not seen on gross examination. Garvin and Work,[1] in 1939, reported 3 cases of coronary embolism, each of which had been ill 1 month or longer with subacute bacterial endocarditis. At autopsy, vegetations were found on the mitral valve in 2 cases and on the aortic valve in 1 case. Emboli were found in the left descending coronary in 2 instances, and in 1 instance in both coronary arteries. In an addendum to the same paper, Work mentioned another case of subacute bacterial endocarditis with vegetations on the aortic valve, and emboli in the left descending and circumflex arteries.

Age:	10–20	1	
	20–30	5	
	30–40	13	
	40–50	5	
	50–60	0	
	60–70	4	
	?	2	
Sex:	Male	22	
	Female	5	
	?	3	
Source:	Endocarditis		12
	Aortic thrombus		7
	Syphilitic aortitis		3
	Ventricular thrombus		2
	Thrombus of coronary artery		2
	Paradoxical embolism		4
Branch:	Left:		
	Main	12	
	Descending	12	
	Circumflex	2	
	Both	4	
	Right:		
	Main	4	
	Descending	1	
Death:	Sudden	23	
	Gradual	6	
	?	1	

An analysis of the 30 cases as to age, sex, source of the emboli, branch of the coronary artery occluded, and the type of death, is given on the preceding page.

Comment. The 3 cases reported are especially interesting in that all were cases of relatively sudden death in colored males with syphilitic aortitis. Textbooks of pathology mention only three complications of syphilitic aortitis; namely, narrowing of the orifice of the coronary arteries, aortic regurgitation, and aneurysm of the aorta. To these we add coronary embolism as one of the possible, though rare, complications.

The pathogenesis of the lesion and mechanism of death appears to be identical in all 3 cases. The gross appearance is characteristic of syphilitic aortitis with moderate amount of atherosclerosis in the first portion of the aorta. The gross impression is supported by histologic studies which reveal active mesaortitis with adventitial round-cell infiltration, destruction of elastic tissue in the media and scar formation. These areas of medial destruction are associated with marked atheromatous changes of the intima, in 1 case the latter is obviously confined to an area just over the medial change. Atherosclerosis in all 3 cases resulted in the formation of mural thrombi attached to the atheromatous plaques. Emboli, from the aforementioned thrombi, resulted in sudden death by complete occlusion of the left coronary arteries in the presence of narrowed orifices of the right coronary arteries. In all 3 cases death was due to acute left-sided heart failure manifested by acute dilatation of the latter and severe pulmonary edema. Death occurred before actual regressive changes in the myocardium had become visible.

Summary. Thirty cases of coronary embolism are analyzed as to age, sex, source of the embolus, branch of the coronary artery occluded, and the type of death. Three new cases of coronary embolism are reported, all of which occurred in colored males. Syphilitic aortitis was the indirect source of the embolus in each case.

We wish to express our appreciation to Dr. Paul Kimmelstiel for assisting us in the preparation of the pathologic material.

Another case has been observed at the Medical College of Virginia in which mural thrombi in the first portion of the aorta were demonstrated as an incidental finding. Death in this case was due to trauma. Microscopic sections revealed that the mural thrombi were situated over atheromatous plaques overlying focal areas of fulminant syphilitic aortitis. The syphilitic aortitis was not noticed grossly and did not involve the commissures of the aortic cusps.

This footnote is added to emphasize the fact that mural thrombi on the beginning portion of the aorta without aneurysmal dilatation are apparently more common than has been hitherto assumed.

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DISSECTING ANEURYSM OF THE AORTA WITH EXPERIMENTAL ATHEROSCLEROSIS.

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It is recognized that dissecting aneurysm of the aorta can heal, and that the newly formed aortic channel can function for years. Patients with such a double aorta can remain symptomless with normal capacity for work. Cases with this type of dissecting aneurysm are relatively rare. As early as 1843 Peacock[6] collected 73 cases of "fully formed dissecting aneurysm." In this group only 4 or 5 cases were of the healed type. In 1934, Shennan[10] analyzed the anatomic and clinical features of 300 cases of dissecting aneurysm of the aorta and pulmonary artery, including 17 cases of his own. In this group, 75 instances are thought by Shennan to be the healed or organizing cylindrical type of dissecting aneurysm; but only about one-half or less of these cases can be regarded as truly healed dissecting aneurysm without subsequent acute tear. One may conclude, therefore, that approximately 1 out of 10 cases of total dissection of the aorta are apt to heal.

The cases to be reported here are unusual, not only because the dissecting aneurysm was completely healed and did not contribute to the causation of death, but also because atherosclerosis was present in the new channel in the wall of the dissecting aneurysm. As a result of the presence of extensive atherosclerosis in the original, as well as in the newly formed aortic walls, the Roentgen ray picture in Case 1 revealed a shadow corresponding to a double aorta.

Case Reports. Case 1.—A. C., a 73-year-old white female, was admitted to the Boston City Hospital on January 11, 1938. She was in good health until the day before admission, when she developed weakness in her right arm and leg, and her speech became thick. Her past history had no significance. She had always been an active person.

On physical examination there was slight facial weakness over the right side. The tongue protruded slightly to the right. The functions of the other cranial nerves were normal. The vessels of the fundi showed arteriosclerosis. The head, neck and lungs were apparently normal. The heart was slightly enlarged to the left; sounds were regular and of good quality. A soft-blowing systolic murmur was heard over the apex. The palpable

arteries were thickened. The arterial pressure was 180 mm. Hg. systolic and 100 diastolic on entry, but subsequently it fluctuated around 150 mm. systolic and 90 diastolic. No organs of the abdomen were palpated. Voluntary motions of the right arm and leg were absent. The reflexes were normal, with the exception of an equivocal Babinski on the right.

The general condition of the patient remained unchanged for 7 days, when she developed a slight respiratory infection, with a temperature of 99.2° F. Four weeks after admission she was allowed to sit up. On March 21, she became acutely ill with signs of bronchopneumonia, and died on April 1.

Examination of numerous specimens of urine failed to reveal abnormal findings. The red blood cell count varied from 4.6 to 4.8 millions; the hemoglobin content from 83 to 109%; and the white blood cell count from 6300 to 9500 previous to the pulmonary infection, rising to 11,400 during the course of the pneumonia. The non-protein nitrogen of the blood was 24 to 33 mg. per 100 cc. The Wassermann and Hinton tests were negative. The phenolphthalein excretion through the kidneys was 46% in 1 hour. The specific gravity of the urine varied from 1.007 to 1.015. The spinal fluid pressure was 140 mm. of H_2O. The total protein content was 32 mg. per 100 cc. The colloidal gold curve was normal. The spinal fluid Wassermann test was negative. The electrocardiogram revealed sino-auricular tachycardia, with left ventricular predominance; *P–R* interval of 0.2 second and *QRS* 0.08 second. T_1 was flat, T_2, T_3, T_4 upright. On January 18, a Roentgen ray of the chest was interpreted as showing a calcified outline of the aorta extending outside of the left hilus region. The lung fields were clear. On March 17, the findings were the same, but, in addition, there was bilateral shadow indicating bronchopneumonia. Following the postmortem examination, however, the Roentgen ray picture was reinterpreted as clearly indicating a double shadow corresponding to the calcified walls of the original and of the dissecting aneurysm (Figs. 1 and 2).

Clinical Diagnoses. Cerebrovascular thrombosis; right hemiparesis; coronary sclerosis, partial heart block; bilateral bronchopneumonia.

Necropsy. Necropsy revealed the following pertinent findings. Both pleural cavities contained some non-bloody fluid and adhesions. In the pericardial cavity there were no adhesions. The heart weight was 300 gm. The orifice and the lumens of the markedly sclerosed and calcified coronary arteries were patent throughout. The myocardium was firm.

Aorta: Above the aortic valve and just below the arch, there was a large opening, which divided the aorta into two lumens of about the same size. This double lumen descended to the right common iliac artery, where the new lumen communicated, through an opening, into the iliac artery. The new lumen lay anteriorly and slightly to the left. Both lumens stood out, and the surrounding walls were rigid and calcified in parts. The double aorta could be cut with difficulty, because of the calcium deposits. The intima, both of the original and of the dissected new lumen, was covered with numerous atheromatous deposits. Several strands 1 to 2 mm. in diameter bridged the lumen of the dissecting aneurysm (Fig. 3).

Lungs: The right lung weighed 850 gm.; the left one was 680 gm. There was evidence of purulent bronchitis, bronchopneumonia and edema. The spleen, pancreas, and liver were essentially normal. The combined weight of the kidneys was 280 gm. with granular surfaces. The cortex was 0.5 to 3 cm. in depth. The red pyramids were somewhat ill-defined. The pelvis and the ureter were normal. The adrenals were normal. The wall of the bladder was thick. The ovaries and the uterus were small.

Microscopic Examination. Aorta: Portions of both vascular channels revealed that the original intima showed fibrous thickening containing fatty macrophages. Below the intima the portion of the muscularis contained

many elastic fibers. The surface of the new channel was covered in places with endothelium, beneath which there was a moderately thick layer of connective tissue, infiltrated with fatty macrophages. The connective tissue showed areas of calcification. A moderate amount of elastic tissue was also present in this portion.

The lungs revealed areas of necrotic material containing numerous microorganisms and neutrophils. The kidneys contained scattered hyalinized glomeruli. The arterioles showed marked intimal thickening. The rest of the findings were not pertinent.

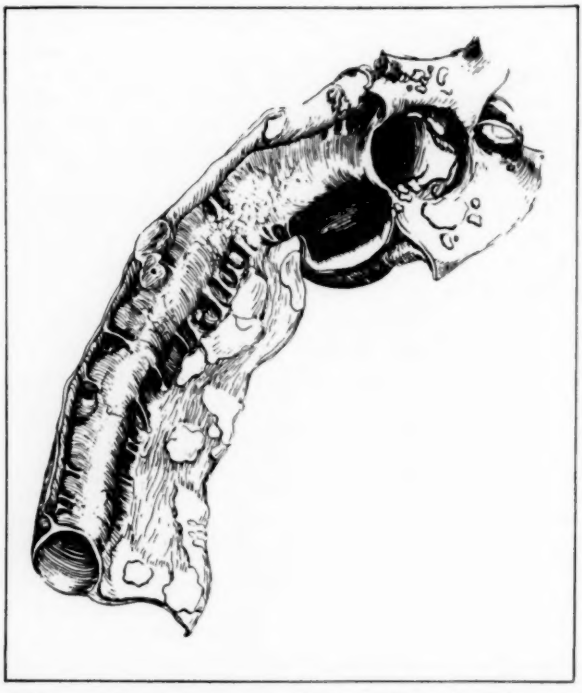

FIG. 3.—Case 1. Gross appearance of the healed atherosclerotic dissecting aneurysm. Atherosclerotic plaques over both the surface of the original aortic wall and the surface of the dissecting aneurysm.

Anatomic Diagnosis. Healed dissecting aneurysm of the aorta and right common iliac artery; marked atherosclerosis with calcification of the original and of the dissecting aneurysm; formation of endothelial islands over the dissected aortic surface; coronary sclerosis; bilateral bronchopneumonia with necrosis.

CASE 2.—A 74-year-old white male (Hosp. No. A76816) was admitted to the New Haven Hospital on November 15, 1937, complaining of severe pain in the left flank of 1 week's duration. He had suffered from "high blood pressure and heart trouble" for 10 or 12 years prior to admission. There had been mild dyspnea on exertion for the past 7 years. The onset of the present illness was 14 months before admission when he was seized by sudden severe "crushing" pain in the chest and abdomen, which had spread within 15 minutes to the left leg, shortly to be followed by numbness

and paralysis of the extremity. All these symptoms subsided within 2 hours. However, he suffered from pain in the left loin for 3 weeks. There was slight ankle edema for a few days. He also noticed that his urine was blood-tinged following the severe attack. This pain was associated with profuse sweating but there was never any true cardiac distress. Following this attack he was symptom-free and able to resume his usual mode of living until November 12, 1937, 3 days prior to admission. At this time there was recurrence of severe pain in the left groin which radiated across the abdomen but not along the course of the ureter. There were several vomiting attacks associated with this pain.

When first examined the temperature was 99.4° F., the pulse 98, the respirations 20, the blood pressure in both the right and left arms was 200/100. The patient did not appear seriously ill. The optic fundi showed normal discs and marked sclerosis of the arterioles; the veins were full and there were a few small patches of exudate on the right side. The neck veins were distended. There was dulness at the left interscapular region and a few coarse râles at both lung bases. The heart was percussed as enlarged, 14 cm. in the fifth space. The A_2 was ringing and accentuated. There was a soft systolic blow at the base. Every second heart beat was followed by an extrasystole. The peripheral arteries were sclerosed and the radial and femoral pulsations were equal. No other significant findings were found either on general or neurologic examination.

The essential laboratory data at this time was as follows: Kahn and Wassermann tests of the blood were negative. Hemoglobin was 91%; red blood cells, 5.2 millions; white blood cells, 8500; smear and differential count normal. The urine was smoky in color; specific gravity, 1.020; acid, albumin 1+; sugar, 0; sediment, 6 to 8 red blood cells per high-power field; 1 to 2 white blood cells per high-power field; few cocci. The non-protein nitrogen was 59 mg. Phenolsulphonphthalein excretion, 35% in 2 hours.

The heart, by Roentgen ray examination (Fig. 4) showed increase in its transverse and anteroposterior diameters as a result of left ventricular enlargement. There was considerable widening of the supracardiac shadow of the thoracic aorta with marked dilatation of the aorta seen in the lateral and left anterior oblique projections. The dilatation involved the ascending aorta, the arch and the upper two-thirds of the descending aorta. Roentgen kymography of the heart showed some flattening of the left ventricular waves which showed irregular notching. The waves of the ascending aorta showed an increase in amplitude. Well-defined waves were seen over most of the large descending aorta which projected to the left. The electrocardiogram revealed sino-auricular tachycardia with left-axis deviation.

The pain gradually disappeared after a few days of bed rest. He was discharged on December 4, 1937, with the diagnosis of hypertensive cardiovascular disease; arteriosclerotic aneurysm of the descending aorta, and benign prostatic hypertrophy. One physician suggested the diagnosis of dissecting aneurysm of the aorta.

The patient was subsequently seen in the hospital in March, 1938, with the complaint of shaking chills due to acute pyelonephritis. The physical and Roentgen ray examinations were essentially the same as on the previous admission. The urine showed a heavy growth of *B. coli*.

He was again admitted in May, 1938, with chills and a history of sudden sharp severe pain over the bladder radiating to the back. The findings were essentially the same as before. The blood pressure, left arm, was 160/90; right arm, 150/80; left leg, 200/110; right leg, 130/100 mm. Hg. An electrocardiogram was identical with the previous study except that the

S–T take-off in Leads 2 and 3 were high and definitely higher than in the previous electrocardiogram.

On August 17, 1938, the patient had a sudden pain in the left chest which radiated down to the left hand. On August 19 he had two shaking chills. On August 26, he developed back pain. A maculopapular eruption appeared over the trunk and extremities. He was admitted to the hospital on August 29, 1938.

The temperature was 100.4° F., pulse 98, respirations 24, blood pressure 160/95. There was a loud apical systolic murmur. The remainder of the examination was non-contributory. The white blood cell count was 20,800; neutrophils, 93%. The urine showed a specific gravity of 1.009, albumin 1+, sugar 0, many white blood cells and rod-shaped cocci. The blood and urine cultures were positive for *B. coli*. The non-protein nitrogen was 291 mg. per 100 cc., the CO_2 24.1 vol. % and the blood chlorides 106. The patient died the day following admission.

Necropsy. Overgrowth of prostate; suppurative cystitis, ureteritis and pyelonephritis (bilateral); necrotizing laryngitis, tracheitis and bronchitis; focal pneumonia and pulmonary abscesses (bilateral); generalized arteriosclerosis; dissecting aneurysm of aorta with endothelialization; cardiac hypertrophy; cortical adenoma of right adrenal.

The aorta was increased in circumference throughout its entire length. This increase was least marked near the aortic valve and most marked at the level of the obliterated ductus arteriosus Botalli.

Two and a half centimeters beneath the mouth of the subclavian artery was a transverse fish-mouth opening in the left lateral wall. This mouth communicated with a false passage or aneurysm. This intimal tear was at the level of the obliterated ductus arteriosus but the tear does not involve this structure. The aneurysm had dissected back around the arch of the aorta for a distance of 2.5 cc., stopping just short of the left subclavian artery. The pocket formed by the dissection was filled by an organized thrombus which extended down to within 0.5 cc. of the opening of the aneurysm (Fig. 5).

The descending portion of the aneurysm extended the entire length of the aorta. It was lined throughout by an intact pearly white layer of tissue resembling endothelium. In a few places the endothelium was elevated by small firm, pearly white atherosclerotic plaques. In the thoracic and upper abdominal portions of the aneurysm were several strands not more than 2 mm. in diameter, which bridged the lumen of the aneurysm. These were quite elastic and were covered by smooth white endothelium.

The aneurysm continued down the right common iliac for a distance of 8 cm. when it again ruptured into the true lumen. The right common iliac secured the greater part of its blood supply from the true aorta, as the aneurysmal opening at the bifurcation of the aorta was but a few millimeters in width. This is indicated by the numeral 1 in Figure 5.

The left common iliac received its entire blood supply from the aortic aneurysm. As indicated by the numeral 2 in Figure 5, the aorta had been sealed off and all that remained of the original communication was a small pouch 1.5 cm. in depth in the common iliac artery.

Microscopic Examination. Sections of the aorta above the point of rupture showed no lesions. At the upper margin of the aneurysmal opening the endothelium and subendothelial tissues bent backwards upon themselves to form the upper lip. The elastic fibers of the media also described this arc but stopped abruptly just above the margin of the lip. The intima merged imperceptibly into an organizing thrombus. Some of the organization tissue was hyalinized. The thrombus was partially covered by a thin layer of flattened endothelial cells which were continuous with the endothelium of the intima of the lip of the aneurysm. The lower margin was very

similar to that of the upper margin. The only variation was the absence of thrombus.

A section through one of the raised intimal plaques in the wall of the aneurysm showed deposits of hyalinized connective tissue with infiltration by fatty macrophages suggesting early atheromatous changes (Fig. 6).

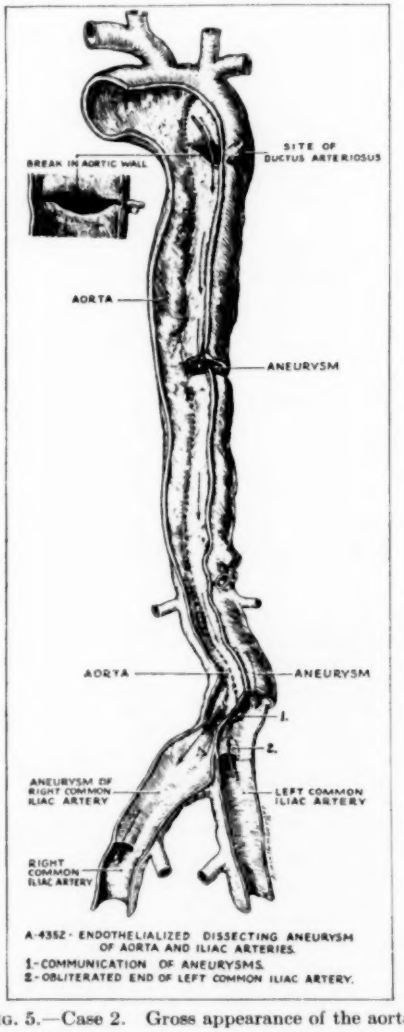

FIG. 5.—Case 2. Gross appearance of the aorta.

All sections of the wall of the aneurysm were covered by intact endothelium. There was a layer of partially hyalinized tissue between the elastic fibers of the media and the endothelium covering the aneurysmal surface. The remnant of the split media in the walls of the aneurysm showed considerable variation in thickness. Neither the adventitia nor the vasa vasorum showed pathologic change.

A cross-section of the right iliac artery showed the aneurysm to be continued into this vessel. The same type of change present in the walls of the aorta was found here. However, there was a well-organized thrombus in the lumen of the iliac artery. This thrombus had been partially recanalized. A portion of the original wall of the iliac artery contained an atheromatous plaque.

The small strands which have been described as spanning the sac of the aneurysm were found on section to be elastic tissue fibers covered by fibrous tissue capped by an endothelial layer. The arrangement of the elastic fibers did not suggest that they represented a portion of a wall of an intercostal vessel. Rather, the arrangement was so irregular as to indicate an origin from a frayed-out strand of elastica from the media of the aorta.

Case 3.—A 51-year-old male was admitted to the Meriden Hospital in coma on March 25, 1939. The patient was said to have become infected with syphilis in 1918. He was not treated for 8 years and subsequently received sporadic treatment. He had been under the care of a local physician for several months because of hypertension. This ranged in the neighborhood of 260 mm. Hg. systolic and 140 mm. diastolic. The patient was said to have had two rather minor cerebral hemorrhages during the 2 years prior to admission. On the day of admission he was found unconscious in his home. He was immediately brought to the hospital.

The temperature was 97° F., pulse 88, respirations 16, and blood pressure 220/150 mm. Hg. The pupils were contracted and the eyes were deviated to the right. There were moist râles heard throughout both lungs. The heart sounds were regular and of fair quality. There were no murmurs. There was a left-sided hemiplegia.

The spinal fluid revealed 623 red blood cells and 52 white blood cells. The spinal fluid Wassermann was negative. The blood Kline reaction was positive. The non-protein nitrogen was 70 mg. per 100 cc. The hemoglobin was 80%; the red blood cells, 4.3 millions; the white blood cells, 10,000. The urine was alkaline with a specific gravity of 1.018, albumin 0, sugar 0, sediment 0. The patient never regained consciousness while in the hospital and died on March 28, 1939, 3 days following admission.

Necropsy. The anatomic findings were massive cerebral hemorrhage in the right frontal lobe involving the corpus striatum and lenticular nuclei and with rupture into the ventricle; focal necrotizing pneumonia with abscess formation (bilateral); benign overgrowth of prostate; dissecting aneurysm of aorta with endothelialization; cardiac hypertrophy; aortical adenoma of adrenal. The heart weighed 510 gm. The wall of the left ventricle measured 15 mm. in thickness while that of the right ventricle measured 4 mm. The heart was otherwise not remarkable.

Aorta: Beyond the origin of the left subclavian artery the aorta had been converted into a double tube. Proximal to this point the lining was elevated by irregular, small, opaque patches of dull yellow atheromatous material. At a point just beyond the slight dimpling produced by the ligament of the ductus arteriosus was the mouth of a sac which had a tubular shape and paralleled the course of the aorta proper. At its dorsal margin the opening of the sac was 0.5 cm. beyond the rim of the subclavian artery (Fig. 7). The sac at first lay dorsal to the aorta but approximately at the point 10 cm. above the origin of the celiac axis, it began to be displaced toward the right. It remained to the right and posteriorly for the remainder of its course. The intercostal and upper lumbar vessels took origin chiefly from the new sac by pinpoint orifices but a few originated in the aorta proper from its dorsal surface which was now represented by the inner anterior lining of the false passage. Cord-like, very elastic bands traversed the lumen of the sac and formed a series of ladder-like rungs. The lining

of the sac in contrast to the faintly yellow appearance and relatively smooth surface of the aorta had a gray color and rough, wrinkled contour. As the celiac vessels were approached, the lumen of the aorta became greatly narrowed, and constricted suddenly 2 cm. below the orifice of the superior mesenteric. The iliac vessels were lined by a smooth, light yellow intima resembling that of the aorta proper. This stopped abruptly just above the bifurcation of the vessels forming a slight ridge that demarcated it from the

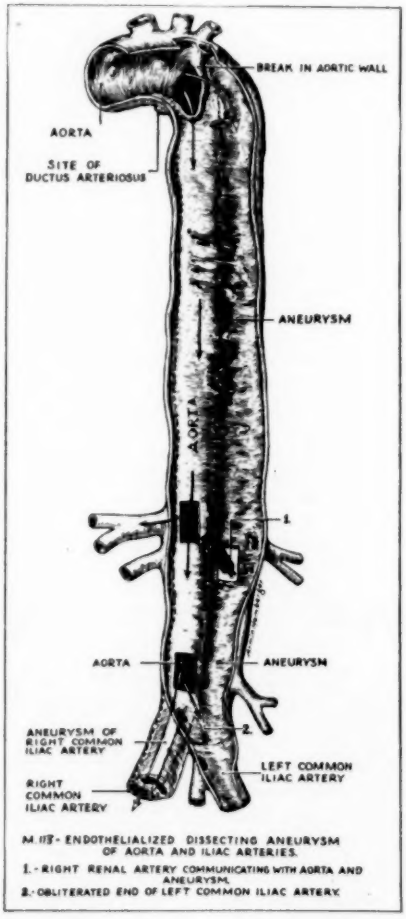

FIG. 7.—Case 3. Gross appearance of the aorta.

rough lining of the false passage. This demarcating ridge was 5 mm. below the linear depression in the medial wall of this sac. The sac had again ruptured into the aorta at this level, namely the bifurcation, but a new wall had formed on the medial side. A probe could be passed into the pinpoint depression, thus demonstrating a communication between the true aorta and the sac at this point. The left internal iliac was a direct continuation of the aorta proper. The external iliac, however, took origin from the

false passage in a linear, almost vertical channel which admitted a probe. The external iliac was lined by several lamellæ of rough, mottled tan and dark red clots, indicating that complete organization had not occurred in the blood clot that covered the rough lining of the sac in this region. Further course of the dissection was not traced beyond a point 2 cm. below the bifurcation of the internal iliac. It is probable, however, that another communication existed between the sac and the true lumen of a branch of the aorta, namely the external iliac, at some lower point that was not observed by the dissection. It is amazing to find that no evidence of bifurcation of a branch of the aorta corresponding to the right common iliac was discovered but that a new wall was established connecting the external iliac with the false sac and completely disestablishing its connection with the original aorta.

Microscopic Examination. A section from the aorta above the rupture showed no lesions. At the upper margin of the aneurysmal opening the intima was intact. The elastic fibers of the media were regularly arranged in their usual fashion. There was no scar tissue or cellular infiltration. The media was broken off abruptly at the mouth of the opening. The endothelium was reflected in a sheet over the fibrous tissue which had been laid down over the broken media while the subendothelial tissue fused into the fibrous mass. The lip still covered by intact endothelium joined the external wall of the aneurysm. This wall was made up of fragments of the torn media embedded in fibrous tissue. The adventitia was made up almost entirely of hyalinized fibrous tissue which was piled up to a considerable height. There were many endothelialized vascular channels in the adventitia and media. This was especially prominent in the angle formed by the lip and the aneurysm wall. One of the vasa vasorum in the angle showed proliferation of the subendothelial tissue with marked encroachment upon the lumen. This may have represented a canalized thrombus (Fig. 8).

The walls of the aneurysm were found to be essentially the same as those in Case 2. Also, sections of the strands bridging the aneurysmal sac were almost identical with those in Case 2 (Fig. 9). Changes identical with those in the walls of the aorta and aneurysm were present in the common iliac artery except that there was no endothelium in this part of the sac of the aneurysm. There was a partially organized thrombus attached to the wall of the aneurysm.

Discussion. The unusual features of the cases described are: 1, the silent course of healed dissecting aneurysm in Cases 1 and 3; 2, the formation of a functionally competent new aortic channel with newly developed endothelium; 3, the presence of advanced atheromatous lesions in the "intima" and in the deeper layers of the wall of the dissecting aneurysm in 1 case and slight lesions in 2 cases; 4, the Roentgen ray picture of the aorta indicating double channel with partially calcified walls in Case 1; and, 5, normal sized heart in presence of "double aorta" of years' duration in Case 1.

The clinical picture of the onset of dissecting aneurysm was typical in Case 2. It is of interest that within 23 months endothelialization and early atheromatous lesions formed over the surface of the new aortic channel. No history of acute tear was obtained in Cases 1 and 3. The etiology was atherosclerosis in Case 1 and arterial hypertension in Cases 2 and 3. In Case 3 severe arterial hypertension and syphilis coëxisted, but the latter played no rôle

FIG. 1.—Case 1. Roentgen ray picture showing a double shadow corresponding to the calcified walls of the original aorta and of the healed dissecting aneurysm.

FIG. 2.—Case 1. Roentgen ray picture showing slightly eccentric view of the bulging dissecting aneurysm with a sharp lineal shadow caused by the calcified wall.

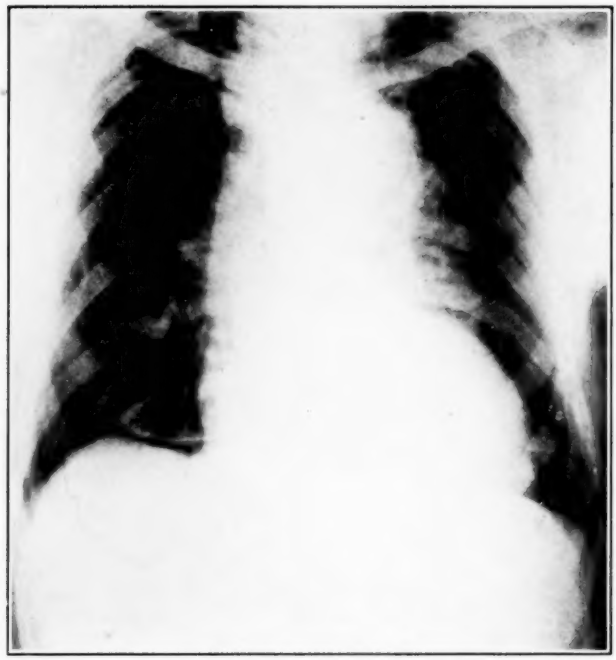

FIG. 4.—Case 2. Roentgen ray picture showing a lighter shadow corresponding to the dissecting aneurysm, with a darker center corresponding to the original aorta.

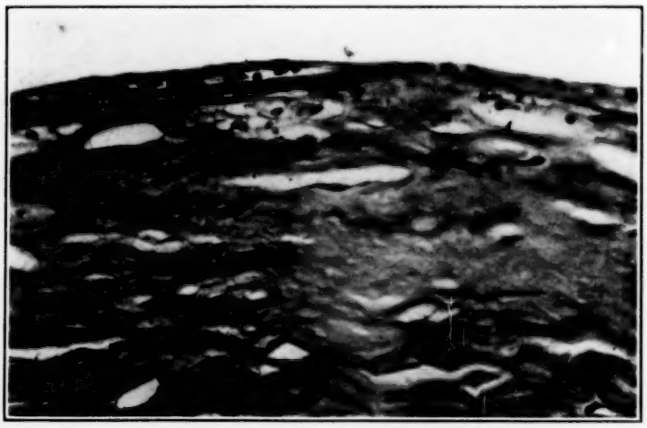

FIG. 6.—Case 2. Early atheromatous change in endothelialized wall of aneurysm. (Hematoxylin and eosin stain, × 225.)

FIG. 8.—Case 3. Vasa vasorum showing proliferation of subendothelial tissue with marked encroachment upon the lumen. (Hematoxylin and eosin stain, × 125.)

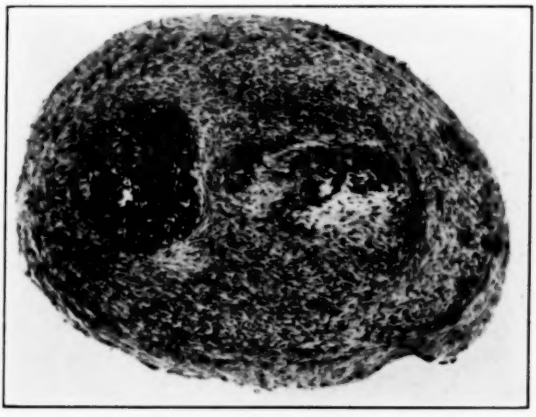

FIG. 9.—Case 3. Cross-section of a strand spanning the aneurysm. (Elastic tissue stain, × 90.)

in the formation of the aneurysm. In describing the clinical characteristics of dissecting aneurysm, one of us pointed out that syphilis, as a rule, plays no part in the causation of dissecting aneurysm; and in 1 case, in which syphilitic and dissecting aneurysms coëxisted, the dissection failed to invade the syphilitic aneurysm.[11a,b] Syphilitic inflammation fuses the layers of the aortic wall. Leary and Weiss[4] indicated that in animals as in man atherosclerosis or hypertension is responsible for dissecting aneurysm. They also pointed out that with the apparent increase in the occurrence of dissecting aneurysm and decrease in that of syphilitic aneurysm, the frequency of the two conditions are approaching one another.

Dissecting aneurysm of the aorta may be compatible with good health over many years. As in the cases here reported, in the truly healed dissecting aneurysm, death is caused by other diseases. The majority of cases reported as "healed" dissecting aneurysm, however, are not actually healed cases, because death is caused eventually by secondary tears or vascular accidents related to the aneurysm. Such is the situation in the case recently reported as "healed" by Roberts.[7] In Shennan's series[10] of 79 cases of old "healed" dissecting aneurysm, in 32 instances a final tear or rupture contributed to death; 34 died from heart failure, and the possibility exists that the presence of dissecting aneurysm contributed to the heart failure. There were 6 cases in which, as in Cases 1 and 3, death was caused by cerebral hemorrhage. In Cases 1 and 3, one cannot state whether the onset was silent, or whether the history failed to reveal the acute episode. Cases have been reported[10] in which the onset was known to have occurred years before death.

The formation of new intima with endothelium in dissecting aneurysm has been described in the literature. In 1887, Bostroem[1] reported in detail the case of a 61-year-old male, who died as a result of cerebral hemorrhage, and in whom postmortem examination revealed a healed dissecting aneurysm of the aorta with an endothelial lining of the intima. Bostroem collected other similar cases for the literature. Among others, MacCallum,[5] in 1909, and Williams,[12] in 1920, each reported a case with formation of the endothelium.

The occurrence of atherosclerosis has been commented upon in the literature in a few cases of healed dissecting aneurysm. As early as 1822 Shekelton considered the possibility that a case of healed dissecting aneurysm was an instance of congenital double aorta because the surface of the new intima looked mildly sclerosed. Bouillaud,[2] in 1847, reported the case of a 36-year-old man in whom marked calcareous degeneration was present over the surface of the new lumen. The most instructive case, however, was that reported by Schilling[8] in 1922. The dissection in this 61-year-old man involved the media of the aorta. The new intima, as in

Case 1, is described as irregularly thick with calcareous deposits. Endothelium was also present in the intima of the new aortic channel. In the case of a 38-year-old man reported by Schnurbein,[9] two attacks of unexplained hemoptysis developed years before the terminal heart failure. Mention is made that atherosclerosis was present to a pronounced degree in the upper, and to a mild degree in the lower portion of the dissecting aneurysm.

The question may be raised as to whether, in these cases, a medial atherosclerosis was not present before the dissection. This may be so in part. In Case 1, however, the extensive flat lesions over the intima were identical in every respect with atheromatous lesions observed in usual instances of intimal and medial atherosclerosis of the aorta. One has to assume, therefore, that the dissecting aneurysm was of long duration, and that factors which led to atherosclerosis in the original aortic wall caused similar lesions in the wall of the dissecting aneurysm.

Kienböck and K. Weiss[3] described a case of dissecting aneurysm in which a Roentgen ray picture showed a fusiform dilatation of the descending aorta with a dark central shadow and a lighter peripheral shadow. Such was the picture in Case 2. Wood, Pendergrass and Ostrum[13] described in detail the roentgenographic features of dissecting aneurysm. They found that extending outward from some point in the aortic arch, there may be an arcuate shadow, which may pulsate. Displacement of the esophagus and trachea may occur. At times a shadow caused by the dissecting channel is present along one or more of the large branches of the aortic arch; this is the most pathognomonic roentgenologic sign. Because of the atheromatous changes in the walls of both aortic channels, the Roentgen ray shadow obtained in Case 1 is particularly prominent and unusual in character.

Summary. 1. Three cases of healed and functioning dissecting aneurysm are reported. Endothelium and atherosclerosis developed over the internal surface of the new aortic channel. The atherosclerosis of the dissected aortic wall was pronounced in 1 of the cases and mild in 2. In none of the cases did the existence of a double channeled aorta contribute to the death of the patient.

2. In a 73-year-old female, with a healed dissecting aneurysm, the Roentgen ray picture revealed a double shadow corresponding to the double channeled aorta. The surface of the new aortic channel was covered with numerous atherosclerotic plaques and endothelial islands.

3. In Case 2 endothelialization and mild atherosclerosis of the dissecting aneurysm developed within 23 months.

4. The histologic structure of the parallel strands bridging the lumen of the new aortic channel indicate an origin from the frayed-out strands of elastica from the media, rather than intercostal arteries.

5. In approximately 10% of the reported cases dissecting aneurysm heals, is not the cause of death, and can be compatible with adequate functional capacity for years.

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COMPLETE OCCLUSION OF THE ABDOMINAL AORTA.

A REVIEW OF SEVEN CASES.

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COMPLETE occlusion of the aorta at its bifurcation has been associated clinically with a well-delineated group of symptoms. The classical syndrome is one of acute onset with severe pain and loss of sensation in the legs, absence of arterial pulsations and rapidly progressive ascending gangrene of the lower extremities with an almost invariable fatal outcome. In a study of the clinical course of 7 patients in whom, at postmortem, complete obstruction of the aorta was found at its bifurcation, we have found that this clinical picture is not a constant one. On the contrary, it appeared in its typical form in only 1 patient. A study of the clinical histories of the others leads us to believe a report of these cases is of interest.

Review of the Literature. Welch,[14] in his classic contribution to the subject of thrombosis and embolism in 1899, reviewed 59 cases of complete occlusion of the aorta. In 1921, Hesse[5] contributed an excellent review of 73 cases, including 2 of his own. In 1922, Wylde[16] added 2 more and Banowitch and Ira,[1] in 1928, gave the total number as 110, including 5 of their own cases. In 1935, Rothstein[13] added 1 and stated that there was a total of 123 cases of complete occlusion of the abdominal aorta.

Thrombosis of the abdominal aorta in infants resulting from infection by way of the umbilical cord has been recorded by Moschcowitz,[11] Wheeler,[15] and Rothstein.[13] Bodeff[3] reported a case of a 10-year-old child who developed an occlusion of the abdominal aorta following diphtheria in the absence of intracardiac thrombi. Menasci[10] reported an instance of occlusion of the aorta complicating influenza. Embolism of the abdominal aorta secondary to subacute bacterial endocarditis was noted by Lop[8] and by Langeron.[7]

The larger number of cases has occurred secondary to chronic rheumatic heart disease, usually in patients with mitral stenosis and insufficiency with auricular fibrillation and associated mural thrombi in the left auricle. The number of such cases is high and instances have been reported by Welch,[14] Hesse,[5] Kulenkampff,[6] Wylde,[16] and others. Multiple vascular thromboses involving the abdominal aorta at its bifurcation secondary to a pedunculated thrombus was reported by Fullerton.[4]

Case Reports. Case 1.—A 65-year-old white male, while receiving physiotherapy for varicose veins, developed an ulcer on the outer aspect of the left leg which progressed, and areas of superficial gangrene appeared despite active treatment. On admission, 7 months later, the pertinent physical findings were: heart not enlarged, regular rhythm, blood pressure 180/110; absent popliteal and femoral pulsations; marked pallor on elevation with redness and pain on lowering of legs; several areas of superficial gangrene over dorsum and lateral aspect of left leg. About 6 weeks later, following rapid progression of the gangrene, he developed bronchopneumonia and died.

The essential findings at autopsy were: heart weight, 350 gm.; marked coronary sclerosis with multiple healed myocardial infarcts. There are atheromatous changes in the arch of the aorta which become more marked in the descending portion; 6 cm. below the origin of the superior mesenteric artery, the aorta becomes occluded by old thrombus extending into both common iliac arteries, the external and internal iliac arteries on both sides, and the left femoral artery. A small portion of the lower abdominal aorta also shows a dissecting aneurysm filled with bright red blood clots.

Case 2.—A 56-year-old white male with intermittent claudication for 15 years, gangrene of the right big toe with healing by conservative treatment two years ago, and recurrence 1½ years later followed by a mid-thigh amputation. Soon afterward, gangrene of the left big toe appeared and quickly progressed upward. The pertinent physical findings on admission were: heart not enlarged, sounds weak, regular rhythm, blood pressure, 200/104; brachial and radial arteries tortuous and hardened; well-healed right mid-thigh stump; left leg cold; an erythematous and puffy area extending one-third the length of the leg; nails of first and second toes absent. A left mid-thigh amputation was performed 2 days after admission, but a cerebral accident occurred 48 hours later and he died in 6 days of bronchopneumonia.

The essential findings at autopsy were: heart weight, 300 gm.; coronary sclerosis and myocardial fibrosis; the aorta shows scattered intimal plaques with ulceration and calcification which increase progressively in the arch and descending portions of the aorta. Just distal to the origin of the renal arteries, an oval mass extends to the bifurcation completely occluding the lumen. This mass is attached to the intima, but is easily stripped, leaving a ragged fibrinous deposit on the intima. The thrombus mass is easily

cut, its periphery is lamellated, the central area is red and contains relatively little fibrous tissue; the thrombus extends into both common iliac arteries.

CASE 3.—A 46-year-old white male developed pain in his lower spine, followed by weakness and inability to walk. Roentgen ray revealed an irregular absorbed area in the third lumbar vertebra which was interpreted as metastatic carcinoma, and was proven by biopsy. The pertinent physical findings were: marked cachexia; heart not enlarged, regular rhythm, blood pressure, 160/100; moderate sclerosis of radial vessels; dorsalis pedis not palpable; marked atrophy of calf muscles with pitting ankle edema. He died in 10 days of inanition.

Autopsy: Carcinoma of pancreas with generalized metastases; heart weight, 300 gm.; marked coronary sclerosis. Aorta is markedly atherosclerotic and inelastic. Its lumen just above the bifurcation is completely occluded by an old gray thrombus which is fixed to the ulcerated intima.

CASE 4.—A 74-year-old white female with known hypertension, auricular fibrillation, and congestive heart failure was suddenly seized with severe pain in both lower extremities and admitted 15 hours later. The essential physical findings were: heart not enlarged, regular rhythm; lower extremities mottled, purple and cold, more marked on the left; no pulsations felt. Patient died the next day following a generalized convulsion.

Autopsy: Heart weight, 280 gm.; marked coronary sclerosis with scattered areas of fibrosis. Aorta shows calcified atherosclerotic plaques in the ascending arch; the lower portion, 3 cm. below the origin of the renal arteries, shows considerable atherosclerosis involving the media and converting it at this level into a rigid tube. This portion and both iliac vessels are filled with red clots adherent to the ulcerated portions of the wall in a few places which can, in general, be removed.

CASE 5.—A 39-year-old housewife with chronic rheumatic cardiovalvular disease for many years and persistent congestive heart failure for 3 years was admitted for numbness of the right foot of 8 days' duration. The essential physical findings were: enlarged heart with systolic and diastolic murmurs at apex, auricular fibrillation, blood pressure, 130/90; enlarged liver; slight pitting edema of lower extremities, dorsalis pedis and popliteal pulsations not felt, hyperesthesia of right foot. She died of a cerebral accident.

Autopsy: Heart weight, 500 gm.; hypertrophy and dilatation of all the chambers with healed rheumatic valvulitis involving aortic, mitral, and tricuspid valves; mural thrombi in both auricles; mild coronary sclerosis. Aorta is elastic, with a few small scattered yellowish plaques. Its lumen at the bifurcation is occluded by a very hard grayish mass which is firmly attached to the wall. This extends to common iliacs, but does not completely occlude their lumens.

CASE 6.—A 38-year-old male with intermittent claudication of 10 years' duration was subjected to a mid-thigh amputation of his left lower extremity for progressive gangrene of 3 months' duration. Seven months later, the toes of his left foot became gangrenous. About 2 years previously the nail of his right index finger was removed for pain, redness and swelling. The essential physical findings were: heart not enlarged, regular rhythm, blood pressure, 160/100; left radial pulse weak, right not elicited; redness and mottling of distal portions of digits of the right hand with thickening of nail-bed; mid-thigh amputation of left lower extremity with well-healed stump; redness and swelling of distal and of dorsum of the left foot with gangrene of first, second and fifth toes. A mid-calf amputation was performed. However, 6 months later, he developed fatal ascending gangrene of the right stump.

Autopsy: Extensive gangrene of left stump and penis, beginning gangrene of right stump; heart weight, 520 gm.; marked hypertrophy and dila-

tation of both ventricles. There is slight coronary sclerosis, healed complete coronary occlusions and an old anterior wall infarct. Aorta is completely occluded by a grayish thrombus which extends from below the renal arteries into the common iliac and right femoral arteries. Microscopically, there is occlusion and recanalization of both right femoral artery and vein.

CASE 7.—A 39-year-old white male, with mild diabetes for 9 years, fatigue and pain in calves in walking for 1 year. During a diathermy treatment, he developed an ulcer on the outer aspect of his right leg which progressed and became gangrenous despite treatment. The pertinent physical findings 9 months later were: heart not enlarged, regular rhythm, blood pressure, 122/83; a discharging punched-out ulcer about the size of a silver dollar on outer middle third of leg.

The ulcer failed to heal; two attempts at skin grafting failed and finally a right supracondylar amputation was performed with satisfactory healing of the stump in 2 months, despite an infection. Three years later, section of the left musculocutaneous nerve was performed for persistent pain in the left big toe. An ulcer formed at the site of incision and broke down repeatedly. Shortly thereafter, he had a sudden seizure of pain in his right forearm with coldness and tingling, loss of radial and brachial pulses, followed by gradual improvement. One year later, he began to experience frequent episodes of precordial pain. Six months before his death, he suffered from an episode of intense pain in the umbilical region which lasted for about a week. Death occurred following a severe anginal seizure.

Autopsy: Well-healed right supracondylar amputation; heart weight, 400 gm.; pericardial adhesions with multiple old coronary occlusion, old healed infarct in posterior wall of left ventricle with a small adherent mural thrombus. Aorta shows mild atherosclerosis; bifurcation is completely occluded by partly organized greenish-red thrombus densely adherent to the ulcerated wall. Both right iliac and femoral arteries are dense fibrous cords; the left iliac and femoral arteries are thickened. There is complete occlusion of the left brachial artery by a recent thrombus.

Discussion. This group of 7 cases was obtained from a review of approximately 5350 autopsies performed at this hospital since 1915, an incidence of slightly more than 1 in 1000. Lueth[9] recently also estimated the incidence as 1 case per 1000 hospital admissions. In addition, we encountered numerous cases of partial occlusion of the aorta which are not included in this study.

This series of 7 cases may be divided into three groups: first, a group of 4 (Cases 1 to 4) with atherosclerosis involving for the most part the aorta and coronary arteries; a second group of 2 cases (Cases 6 and 7) with diffuse vascular disease involving the upper extremities in one and the veins in the other; a third type of 1 case (Case 5) with chronic rheumatic cardiovalvular disease with mitral stenosis and insufficiency, auricular fibrillation, and a ball-valve thrombus of the left auricle.

The first group, 3 males and 1 female, ranged in age from 46 to 80. Of these, only 1 (Case 4) presented the classical clinical syndrome of complete occlusion of the aorta on admission. In another of this group (Case 3), there was nothing in the clinical course to suggest involvement of the aorta; but at postmortem the bifurcation was completely occluded by an old gray thrombus. Of interest in the other 2 (Cases 1 and 2) is the rapid progress of gangrene with speedy involvement of the opposite extremity.

In the 2 cases with diffuse vascular disease (Cases 6 and 7), the aortic involvement seems to be a mere incident in the course of a progressive disease involving the entire arterial tree with an arteritis in one and venous involvement in the other. The last case (Case 5) resembles in almost every detail those described in the literature, where complete occlusion at the bifurcation of the aorta occurred as a complication of chronic rheumatic heart disease with arrhythmia and intracardiac thrombi.

Complete occlusion of the aorta may occur by several means: I. Embolism from: A. Thrombus higher in aorta, *i. e.*, aortic aneurysm or thrombosed arteriosclerotic plaque. B. Left side of heart: (1) mural thrombi secondary to myocardial infarct; (2) valvular vegetations—subacute or acute bacterial endocarditis. C. Pulmonary veins—thrombosis. D. Right side of heart—by way of foramen ovale (paradoxical embolism). II. Thrombosis *in situ* associated with: A. Ulcerated atherosclerotic plaque. B. Dissecting aneurysm of aorta. C. Arteritis of abdominal aorta. D. Congenital narrowing of aorta. III. Infection: A. By way of umbilical vessels—in infants. B. Associated with infectious diseases such as diphtheria and influenza.

The differentiation of embolism from thrombosis as a cause of occlusion of the aorta is difficult. Welch[14] pointed out that a decision as to the existence of one or the other is often guess work. As Neuhof[12] pointed out, the mode of onset does not indicate whether one is dealing with embolism or thrombosis, since thrombosis may set in with severe acute symptoms and embolism may produce only intermittent claudication for a long time or no symptoms depending upon the size of the vessel and the collateral circulation.

The generally accepted clinical picture of occlusion at the bifurcation of the aorta is that of a sudden onset with severe acute symptoms and violent pain. Sensory loss, areflexia, numbness, coldness, pallor, cyanosis, and loss of pulsations in the lower extremities develop. Paraplegia and shock rapidly follow with ascending gangrene eventuating rapidly in death. However, most striking in this group of 7 cases is the marked departure from the classical clinical syndrome in 6 cases (Cases 1 to 3, 5 to 7). Indeed, in 1 (Case 3), there was nothing in the clinical history that might be related to the aortic occlusion. One must postulate the presence of an efficient collateral circulation which assumes the responsibility for the vascular integrity of the lower extremities. In such instances as Case 3, the abdominal aorta is physiologically not an "end" artery. According to Bickham,[2] the following collateral areas play an important part in maintaining an adequate blood supply to the lower extremities. There is the internal mammary artery above with the deep epigastric below; the inferior mesenteric above with

the internal pudic below; also, branches of the lumbar arteries above with branches of the internal iliac below.

There are several facts in the clinical course of the other cases which, in retrospect, should have suggested a centrally located occlusive process in the aorta in addition to isolated vascular disease of the extremities. For example, after the onset of gangrene in one big toe in Case 2, progression was so rapid and involvement of the other extremity occurred so early that within 5 months it was found necessary to perform mid-thigh amputation bilaterally. In a second instance (Case 6), 6 months after a successful amputation, rapidly ascending gangrene began at the stump and resulted fatally in a short time. In those cases, we believe the rapid course with early bilateral involvement, recurrence of gangrene at the stump with progression were probably due to the presence of the complete occlusion of the aorta at its bifurcation.

Summary. 1. Seven cases of complete occlusion of the aorta at its bifurcation, observed during the course of 5350 autopsies, are presented.

2. Of these, 4 were patients with atherosclerosis of the coronary arteries and aorta, 2 with diffuse vascular disease involving the venous system, and 1 with chronic rheumatic cardiovalvular disease, auricular fibrillation and a ball-valve thrombus of the left auricle.

3. Clinically, the classical picture usually associated with complete occlusion of the aorta at its bifurcation occurred in 1 case. In the remaining 6, the clinical variations were probably due to: 1, differences in the rate of progression of the occlusion; 2, the presence of an adequate collateral circulation; and 3, subsequent development of a more efficient anastomotic circulation.

4. In 2 cases of peripheral arteriosclerosis, a rapid and progressive course with early bilateral involvement and the recurrence and progression of gangrene of the stump should have suggested the existence of an associated occlusive lesion of the aorta.

5. The difficulties inherent in making the differential diagnosis between thrombosis and embolism as the cause of complete occlusion are discussed.

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THE VALUE OF THE ETHER CIRCULATION TIME IN THE DIAGNOSIS OF RIGHT HEART FAILURE.*

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SINCE the early work of Blumgart and his associates,[3,4] increasing interest has been manifested in methods for estimating the speed of the circulating blood. Blumgart[2] extensively reviewed the entire subject in 1931, and a bibliography up to 1938 is included in one of the author's papers.[1]

For the past few years we have studied some of the procedures advocated in the measurement of the velocity of blood flow. One method to which we have paid particular attention is the ether arm-to-lung circulation time. As originally described by Hitzig,[8a,b] it consists of the intravenous injection of 5 minims of ether and 3 minims of saline. The end point is the subjective or objective perception of ether in the upper respiratory passages. Since the ether circulation time is an accurate index of the functional activity of the right heart unit, this procedure when done in conjunction with the arm-to-tongue circulation time, permits the differentiation of right and left heart failure.

We propose to give some of our observations obtained in studying the ether circulation time, and consider the value of this procedure in the differential diagnosis of cardiac failure.

Method. The test originally suggested by Hitzig[8a,b] and modified by Miller[12] was used. Patients were made to lie in bed until pulse and respiratory rates reached basal levels. After the performance of the calcium gluconate†[1,7] arm-to-tongue time, the needle was left *in situ* and the ether circulation time determined. A mixture of 5 minims of ether and 5 minims of saline is injected into an antecubital vein; the end point is the perception of ether in the upper respiratory passages. Frequently the patient will gasp, cough or grimace in indicating the presence of the ether. In addition, the test is objective as well as subjective, for the observer close to the patient recognizes the odor as promptly as does the patient.

All circulation rates are recorded in seconds and timed by stop-watch from the beginning of the injection to the end point.

Results. The velocity of blood flow from arm to lung was determined in 329 patients (see Table 1).

* Aided by grants from The Jewish Hospital Research Fund and Sandoz Chemical Company.

† Brand of calcium galactogluconogluconate—courteously supplied by Sandoz Chemical Company.

TABLE 1.—SUMMARY OF DATA ON ETHER CIRCULATION TIME.

	Patients.	Determinations.	Range (sec.).	Average (sec.).
Normal	184	206	3.3–9.4	5.8
Pulmonary Disease	26	27	2.5–10.0	6.1
Cardiac disease:				
Without failure	38	40	4.0–8.5	5.8
With failure	52	58	7.0–19.0	10.2
Hyperthyroidism	20	26	3.6–8.0	4.6
Hypothyroidism	9	11	4.0–9.0	6.6

The normal ether arm-to-lung time obtained by Hitzig[8a,b] and others[1,10] ranged from 3.5 to 8.0 seconds, with an average of 5.6 seconds. In 184 patients with no known cardiac or pulmonary disease, the ether time ranged from 3.5 to 9.4 seconds with an average of 5.8 seconds. In only 6 of the 184 cases was it prolonged beyond 8 seconds, and in none beyond 9.4.

One of the most important applications of this test is in the differential diagnosis of dyspnea. Ether times were therefore done on 26 patients having definitely proven pulmonary disease. These included cases of bronchitis, broncho- and lobar pneumonia, empyema, lung abscess, tuberculosis, emphysema, bronchiectasis and bronchial asthma. In only 2 of 27 determinations was the ether time prolonged beyond 8 seconds. Both of these prolonged right heart times occurred in elderly men with arteriosclerosis and pulmonary tuberculosis.

The ether time was determined on 90 patients with some form of heart disease. It is in this group that the greatest deviations from normal occur. In the group as a whole, the ether time varied from 4 to 19 seconds with an average of 8.5 seconds. But if we divide these cases into two groups, those with and without signs or symptoms of cardiac failure, more striking figures are obtained. In those patients in whom no evidence of cardiac failure could be obtained, the ether times were normal. But in those exhibiting some clinical basis for a diagnosis of cardiac failure, the average ether arm-to-lung time was 10.2 seconds.

A number of determinations were also done on patients with hypo- or hyperthyroidism. As seen in Table 1, they fall within normal limits.

Discussion. Isolated right heart failure is a rarity. As pointed out by White[16] and others, the commonest cause for right ventricular failure is failure of the left side of the heart. A normal ether time naturally does not preclude the existence of left ventricular insufficiency. But in those cases in which right heart failure must be differentiated from mediastinal or pleural tumor, chronic pulmonary disease, nutritional edema or venous obstruction, the ether arm-to-lung time may yield valuable information.

Hitzig[8a] found that cases of pleuropulmonary disease exhibited normal ether times, and Oppenheimer and Hitzig[13] showed that uncomplicated pulmonary insufficiency is usually attended by nor-

mal circulatory measurements. Weiss and Kleinbart[15] and others[6,13] used the ether test to help differentiate cardiac and pulmonary factors in chronic pulmonary disease, and Plotz[14] used circulation time tests in distinguishing asthmatoid heart failure from bronchial asthma. Hussey[9] found that the ether time was prolonged only in complete heart failure, in isolated right heart failure, and in isolated incipient right heart failure. Certainly our observations have lent confirmatory evidence to these findings.

Recently a number of observers[5,11] have suggested the use of paraldehyde in determining the right heart time, in preference to ether. The procedure and end results are essentially the same.

Conclusions. 1. Ether arm-to-lung circulation times were done on 329 patients.

2. The ether time in 184 normal patients ranged from 3.5 to 9.4 seconds, with an average of 5.8 seconds. Only 6 of the 184 patients had times beyond 8 seconds.

3. In 26 patients with proven pulmonary disease, the ether time ranged from 2.5 to 10 seconds. The average was 6.1 seconds, and only 2 of the 26 patients had times beyond 8 seconds.

4. In 90 patients with cardiac disease, the ether time ranged from 4 to 19 seconds. The average in patients without heart failure was 5.8 seconds, in those with heart failure 10.2 seconds.

5. The ether test is of definite value in the diagnosis of right heart failure, and its differentiation from conditions simulating right heart failure.

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HUMAN STERNAL BONE MARROW IN HYPERTHYROID AND MYXEDEMATOUS STATES.

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Although much has been written regarding changes in the peripheral blood in thyroid dysfunction, the reports of the condition of the bone marrow in these studies are extremely scanty. For this

reason our finding of consistent hyperplasia of sternal marrow in the thyrotoxicosis and hypoplasia in long-standing myxedema is recorded.

Until 1939 the only reported observations found on human marrow in hypothyroidism were limited to postmortem studies of Langhans.[6] Maresch,[11] Dieterle,[3] and Stoccoda,[15] all of whom found the fatty marrow increased at the expense of the red. In hyperthyroidism, Askanazy[1] stated that this condition usually causes no notable changes in the bone marrow. Renzi and Lenzi[13] recorded differential cell studies on sternal marrows from 17 hyperthyroid and 2 myxedematous individuals. A preliminary report of these observations was published, but no quantitative changes in the nucleated cells of the aspirated marrow were mentioned.

Postmortem marrow findings in thyroidectomized rabbits were reported by Esser,[4] Tatum,[16] and Kunde, Green and Burns,[5] and indicate that the marrow is fatty and hypoplastic. Histologic evidence of increased marrow activity in rabbits stimulated by thyroid was found by Kunde, Green and Burns,[5] and Power.[12]

The numerous publications dealing with the peripheral blood in thyroid disturbances are reviewed by Bomford,[2] and he adds some careful observations on anemia in myxedema. In reporting these Bomford states that marrow biopsy was not in use at the time, and he questions the value of the procedure because all indications point to a diminished total amount of marrow that is qualitatively normal in myxedema.

Methods. The method used for obtaining and studying these marrows was devised by Schleicher and Sharp[14] and a more recent article by Limarzi[8] describes the apparatus and procedures in detail. To state it briefly: 1 cc. of sternal marrow is aspirated through a dry 16-gauge needle into a dry syringe and placed immediately into a paraffin-lined tube containing heparin as an anticoagulant; the marrow is transferred to a Wintrobe tube; centrifuged 5 minutes at about 2000 r.p.m.; the layers into which the marrow separates (erythrocytes, nucleated cells, plasma, and red and yellow fat) are recorded; the nucleated cell layer is mixed with an equal volume of plasma in a paraffin chamber; and films of this mixture are made, stained with May-Grunwald-Giemsa dyes, and studied microscopically for cell distribution and types.

Studies of the peripheral blood, drawn immediately before the sternal marrow, is made in all cases and this includes erythrocyte, leukocyte, platelet and hemoglobin determinations, mean corpuscular volume, hemoglobin and hemoglobin percentage, sedimentation rate, icteric index, reticulocyte count and differential leukocyte count.

Over 1200 simultaneous studies of the peripheral blood and sternal marrow, like the above, have been done by members of the hematologic section of the College of Medicine of the University of Illinois, and the findings in thrombocytopenic purpura were recently reported by Limarzi and Schleicher.[9]

While the collection and study of these marrows was in progress, a patient with hyperthyroidism was examined more or less by

accident, the blood and bone marrow work having been done because of the discovery of an enlarged spleen. The aspirated sternal marrow, in this case, was found to contain a higher percentage of nucleated cells (myeloid-erythroid layer) than had been found in any conditions, other than the chronic leukemias and relatively severe anemias, excluding certain aplastic types and those due to acute hemorrhage. Stained smears of these cells showed that the increase was in the myeloid series, and peripheral blood work showed that this increase in the marrow was not reflected peripherally by an increased leukocyte count, as is usually the case in chronic myelogenous leukemia. Subsequently, 11 other patients with hyperthyroidism were found to have comparable marrows, and the findings are recorded below.

Shortly following this, the marrow of a patient with untreated spontaneous myxedema was found to contain a low myeloid-erythroid layer, as compared to "normal," and it was decided that all cases of thyroid dysfunction that could be secured would be studied hematologically.

To date, we have collected and studied 19 with thyrotoxicosis and myxedema, from the Research and Educational, and Cook County Hospitals, and 18 "normal" volunteers and preoperative hernia patients. The myeloid hyperplasia of thyrotoxicosis and hypoplasia of hypothyroidism, as compared to the percentage of nucleated cells found in "normal" aspirated marrow, has been consistent enough in this small number of cases to make it seem worth while reporting.

Chart 1 shows the myeloid-erythroid layer in per cent of total bone marrow aspirated from the sternum in 12 cases of hyperthyroidism, as compared with "normal" and hypothyroid averages.

The clinical symptoms and findings of hyperthyroidism were clean-cut in all except Case 9, who had a coëxisting severe diabetes. Case 11 was one of thyrotoxicosis, which followed the ingestion of 5 gr. of thyroid daily for about 3 months. The thyrotoxicosis in the rest of the cases was thought to be due to glandular dysfunction. All had several tests of basal metabolic rates previous to the aspiration of marrow, which varied from +34% to +150%, with the exception of the first which was +15%. The myeloid-erythroid layer in this case is just above the 6.2% normal average. Beyond this, there does not seem to be much correlation between the height of the layer and the metabolic rate, although most of those with high rates were in higher levels. The rates and levels are as follows:

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
B.M.R.(%)	15	45.0	62.0	34	37.0	45.0	37	38	75	65.0	150.0	72
M.E. layer(%)	7	8.4	9.5	10	11.6	12.5	14	14	16	16.8	20.5	22

Chart 2 shows the level of nucleated cells in the aspirated sternal marrow of 7 patients who were clinically hypothyroids, and had

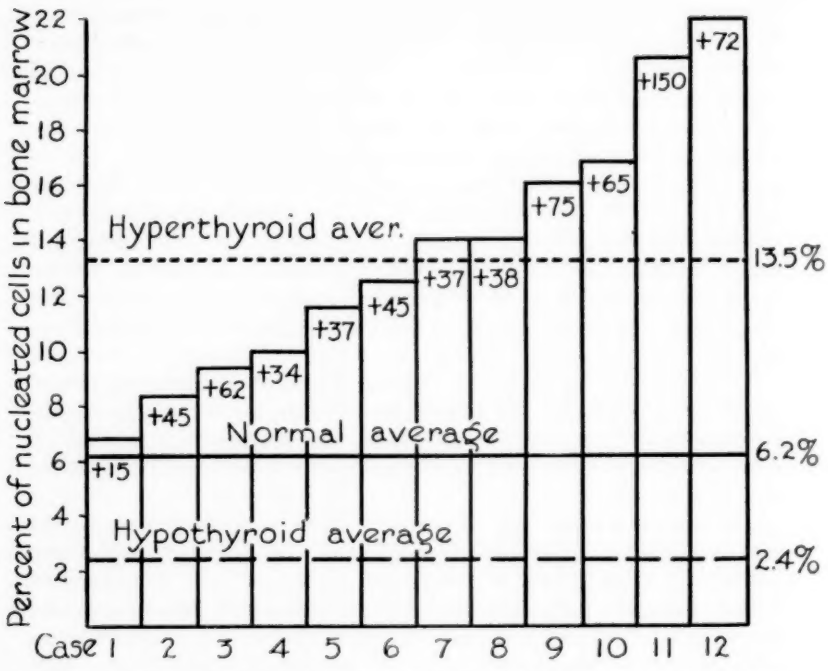

CHART 1.—Per cent of nucleated cells in hyperthyroid bone marrow with metabolic rates.

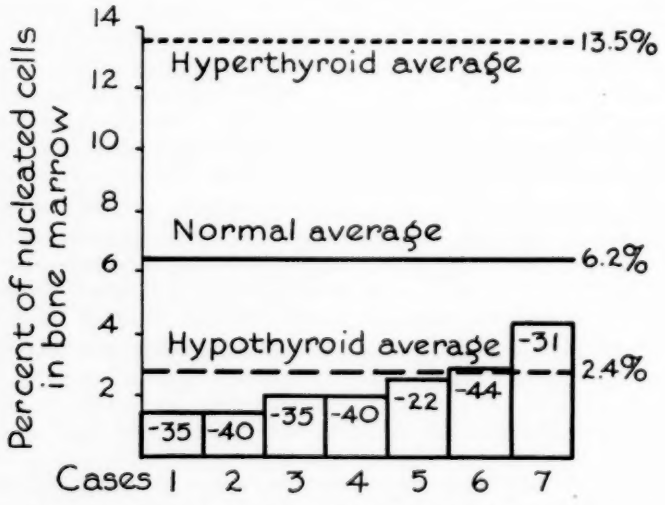

CHART 2.—Per cent of nucleated cells in hypothyroid bone marrows

repeated low basal metabolic rates. These varied from −22% to −44%. The patient with the −22% rate was a case of post-operative hypothyroidism, all the rest were spontaneous myxedemas. The rates and levels are as follows:

	1.	2.	3.	4.	5.	6.	7.
B.M.R. . . .	−35.0%	−40.0%	−35%	−40%	−22.%	−44%	−31.0%
M.E. layer . .	1.5%	1.5%	2%	2%	2.5%	3%	4.5%

Chart 3 shows the results obtained from the marrows of 18 "normals." The patients, from whom these marrows were obtained, were chosen because they had no complaints other than the presence of hernia in some, and because studies of the peripheral blood revealed figures that fell within generally accepted normal limits.

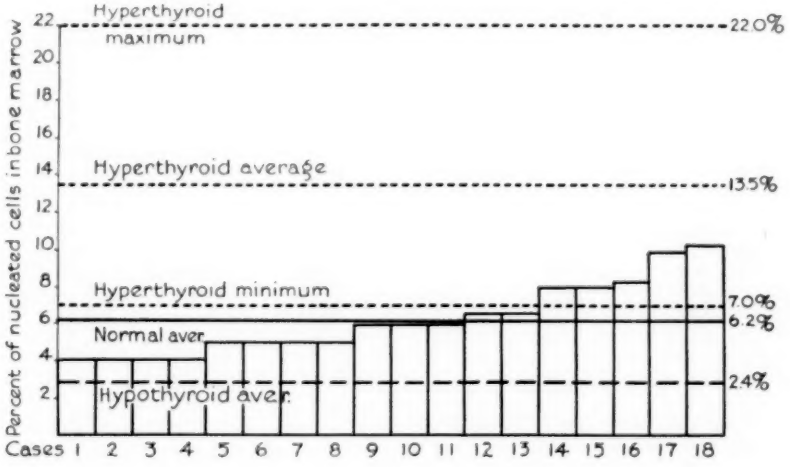

CHART 3.—Per cent of nucleated cells in normal bone marrows.

Peripheral blood studies were done on the patients grouped above as hyperthyroids, "normals," and hypothyroids. The average figures for each of the three groups are tabulated in parallel columns below to facilitate comparison.

From Table 1 it can be seen that in this series the difference between the hyperthyroids and "normals" in peripheral blood findings is not marked. The hyperthyroids show a slight anemia, which depends entirely on a numerical reduction in erythrocytes, there being no significant difference in mean corpuscular volume or hemoglobin concentration between these and the "normals." Differential counts show a slight lymphocytosis in the hyperthyroids, as compared with the "normal" findings, but this is not large.

The hypothyroids show a moderately severe anemia which is, as with the hyperthyroids, due mainly to a reduction in number of the

erythrocytes. The mean corpuscular volume is normal, and the hemoglobin concentration is slightly reduced. The leukocytes quantitatively and qualitatively are practically the same as found for the "normals." One case, the postoperative thyroid (Chart 2, Case 5), showed a moderate macrocytosis (106 cu. microns) which returned to normal (86 cu. microns) after 5 months of thyroid therapy and the return of the marrow to "normal" (Chart 4). This macrocytosis was offset by 1 case (Chart 2, Case 4) in which a microcytosis (67.5 cu. microns) and typical iron-deficiency anemia was found. According to reports of the attending physician, the anemia in this case has cleared up after 4 months of iron and thyroid therapy. The rest of the hypothyroid series showed a reduction in erythrocytes, with no tendency toward macrocytosis or microcytosis.

TABLE 1.—AVERAGE PERIPHERAL BLOOD FIGURES.

	Hyperthyroid.	"Normal."	Hypothyroid.
Hemoglobin, gm./100 cc., Newcomer	13.4	14.7	9.4
Erythrocytes, millions/c.mm.	4.75	5.01	3.51
Leukocytes per c.mm.	8050	7700	7800
Hematocrit (E)	41.2%	45.0%	30.6%
Hematocrit (W.B.C.)	Less than 0.5%	Same	Same
Mean corpuscular volume in cubic microns	86.7	90.0	88.6
Mean corpuscular hemoglobin in micro-micrograms	28.2	29.5	26.8
Mean corpuscular hemoglobin concentration	32.5%	32.7%	30.3%
Reticulocytes	Less than 1%	Same	Same
Icteric index	4 units	6 units	8 units
Neutrophils	50.0%	60.4%	55.6%
Eosinophils	1.8%	2.8%	2.0%
Basophils	0.2%	0.9%	0.8%
Lymphocytes	41.6%	28.9%	35.2%
Monocytes	6.4%	7.0%	6.4%

Chart 4 shows the change found in the aspirated sternal marrow following therapy with varying amounts of desiccated thyroid and thyroxin in 5 cases of myxedema. The dates of aspiration and basal metabolic rates at the time are shown on the chart. The amount and type of thyroid medication is also shown on the chart, except for the first case, and this will be given in detail below.

Case Abstract. The first case of Chart 4 is a male, aged 29, first seen, May 5, 1938, in the hematologic outpatient section of the university clinics. He came directly with a letter from his doctor, stating that he had a peculiar anemia that did not respond to liver or iron, but was, in his doctor's opinion, an atypical pernicious anemia. His complaints at this time were weakness, inability to keep up with things physically or mentally, weight loss, and intolerance to cold. All, except the weight loss, had been gradually increasing for the past year, until 7 weeks previously, when he had become so sluggish that he could no longer keep up with the assembly line, and had been obliged to leave his job of motor assembler in a farm implements plant. The weight loss had been in the past 8 weeks when he had lost his appetite completely. His wife, who accompanied him, told the examiner confidentially that he had periods of mental confusion lately that frightened her, and that she had signed a complaint, and that unless he improved, the trial for commitment would be held soon. Past and family history were

essentially negative. Physical examination revealed a well-nourished white male 6 feet 2 inches in height, and weighing 175 pounds, who was coöperative, but ponderous and slow in answering questions. He had a dry, thick, pale and cold skin, and a heavy face with infra-orbital puffiness. His pulse was 72, and blood pressure 110/90. He was admitted to the hospital with a diagnosis of spontaneous myxedema, which was borne out by subsequent laboratory findings on May 6, 1938 of B.M.R. −35, blood cholesterol 434 mg. per 100 cc., low voltage E.K.G., and subsequent response to thyroid medication. The metabolic rates and the dates are listed on Chart 4. One grain of desiccated thyroid was given daily from May 17 to 26. Two grains daily were given from May 26 to June 1, then 3 grains daily from June 2 to October 3, at which time the dose was reduced to 1 gr. daily until December 3. Since December 4 he has taken 2 gr. daily. His mental and physical difficulties disappeared rapidly when a dose of 3 gr. daily

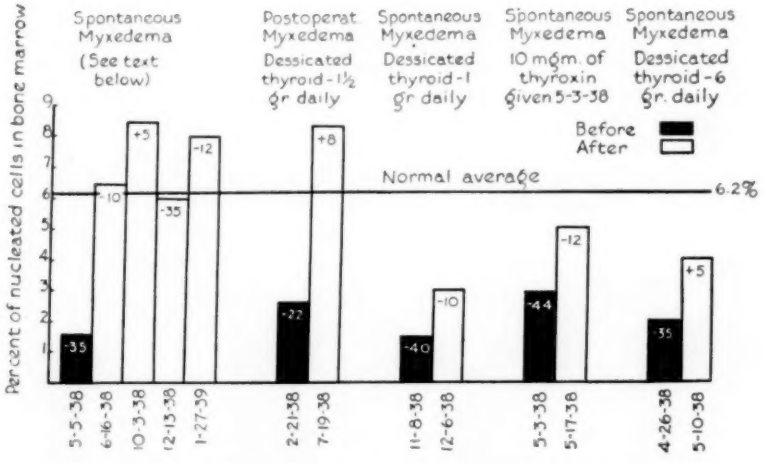

CHART 4.—Effect of thyroid therapy on hypothyroid marrow.

was reached. The response of his metabolic rate and aspirated bone marrow is shown on Chart 4. His blood cholesterol fell to 220 on June 3, 185 on June 8, and 172 on June 21. His peripheral blood findings have changed but little in comparison to the change in his appearance and condition. The first findings (erythrocytes, 4.16 millions; hemoglobin, 12.5 gm. per 100 cc.; mean corp. vol., 89 cu. microns; mean corp. hb. conc., 35%) on May 5, after slight initial fall in red count and hemoglobin, rose gradually until December 13, when they were: erythrocytes, 4.97; hemoglobin, 13.25 gm.; mean corp. vol., 80 cu. microns; mean corp. hb. conc., 33%.

The other treated myxedemas were females, and varied in age from 30 to 54 years. The response of their metabolic rates and the changes found in marrow aspirated from the sternum are also shown on Chart 4.

Chart 5 represents the changes found in sternal marrow and metabolic rates after stopping the ingestion of desiccated thyroid in the first case, and subtotal thyroidectomy done for the relief of thyrotoxicosis in the others. The changes in this group are less

striking than those found in the hypothyroid cases above, and even from the small number studied, one receives the impression that the readjustment downward of the hyperplastic marrow is much slower than the rise of the myxedematous marrows on treatment. The first case in this group is one of thyrotoxicosis that was induced in a 22-year-old female by self-medication for obesity. She had taken 5 gr. of desiccated thyroid daily for about 3 months, and when admitted to the hospital had a B.M.R. of +150% (average of several tests after sedation), warm, moist skin, tachycardia, tremor, and was so unstable that she was placed in the Psychopathic Institute for several weeks to prevent her from doing harm to herself. The changes in her peripheral blood were minor compared to those

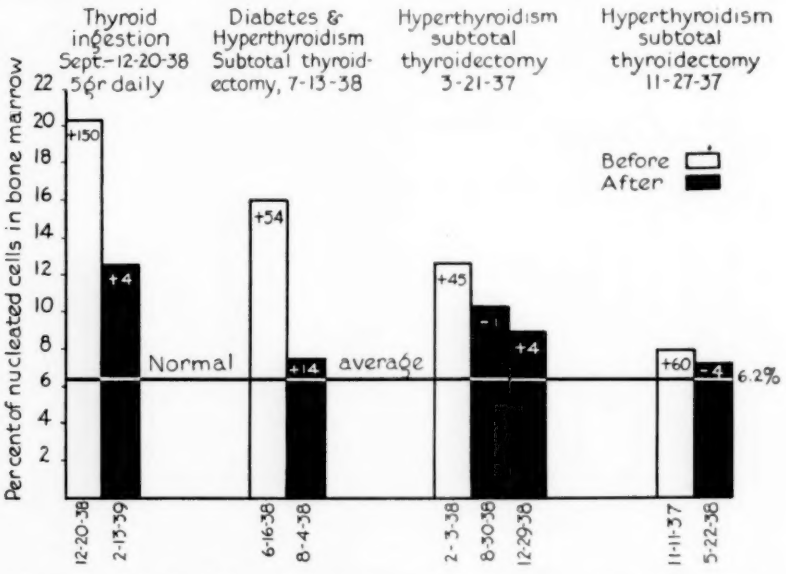

CHART 5.—Reaction of hyperthyroid marrow to withdrawal to thyroid stimulation.

found in the aspirated marrow. In the following list the first figure represents peripheral findings on Dec. 13, 1938, when her B.M.R. was +150%, and the second, the findings on Feb. 13, 1939, when her B.M.R. was +4%: erythrocytes, 5.68 and 5.39; hemoglobin, 14.25 and 13.8; mean corp. vol., 75 and 82.5; mean corp. hb. conc., 34% and 31%.

In evaluating the results represented by the above charts, it must be remembered that the possibilities for quantitative error are great. The percentage of nucleated cells in the bone marrow shown on the charts is accurate only for the bit of marrow aspirated from the sternum at that time. Dilution with sinusoidal blood must lower the myeloid-erythroid layer occasionally. There have been times

during more than 1200 aspirations that a large sinusoid has obviously been entered, and the aspirated material differs but little from peripheral blood. When this has happened, and such occasions have been surprisingly few, repeated aspiration at a different level of the sternum has been done.

Also it must be remembered that the activity of the sternal marrow, as shown by the percentage of nucleated cells in an aspirated sample, is not a definite indication of the activity in other bones, or of the total mass of active marrow in the body. As has been shown by postmortem examination of the bone marrow of cretins and older individuals, while the sternum, ribs and vertebræ usually contain active red marrow, the marrow of the long bones may be entirely fatty. Bomford,[2] from the scanty evidence available, says that hypoplasia of the active marrow in myxedema is probably on a quantitative basis, that is, that the total active red marrow in the body is reduced in amount, but normal in quality. The findings of this study indicate that the active marrow itself differs from the normal. (See Chart 2.)

Bomford in the same article explains the anemia of myxedema as possibly due to an atrophy of the erythron in adaptation to slowed metabolic processes and diminished oxygen want. The possible reverse of this process in hyperthyroid states is an interesting speculation. Bomford mentions that it seems possible that in polycythemia vera, the increased metabolism is the primary disorder, and the increased erythrocyte formation secondary to this. It was such reasoning that prompted Limarzi, Keeton and Seed[10] to try thyroidectomy in polycythemia vera with some success.

The incidence of polycythemia vera is low when compared with the total incidence of other conditions with increased metabolic rates. Certainly glandular dysfunction without marked blood changes is much more common. Even chronic myelogenous leukemia with a marked rise in the metabolic rate is reported with greater frequency. The bare possibility that the myeloid hyperplasia of the marrow reflected in the peripheral blood in the last-mentioned disease is secondary to the increased metabolism, instead of the cause of it, should not be overlooked. Further speculation might be advanced that a hyperthyroid state is an initiating factor in the development of chronic myelogenous leukemia in susceptible individuals.

The hyperplasia found in the aspirated sternal marrows of 12 individuals with increased metabolic rates (Chart 1) differs markedly from the hyperplasia commonly found in the anemia of iron deficiency, chronic blood loss, hemolytic jaundice and pernicious anemia. Study of stained smears of the nucleated cells shows that the hyperplasia in the anemic cases is due to an increase in the precursors of red cells, normoblasts, and pronormoblasts, while in the hyperthyroid cases, the hyperplasia is myeloid, with an increase in metamyelocytes, myelocytes, promyelocytes, megakaryocytes.

Summary. 1. Sternal marrow aspirated from 18 "normal" individuals contained an average of 6.2% nucleated cells.

2. Sternal marrow from 12 individuals with hyperthyroidism contained an average of 13.5% nucleated cells, about $2\frac{1}{5}$ (217%) times the "normal" finding.

3. Sternal marrow from 7 individuals with hypothyroidism contained an average of 2.4% nucleated cells, a little more than one-third (38%) of the "normal" average.

4. Five cases of hypothyroidism treated with desiccated thyroid or thyroxin, and subsequently studied, showed a marked rise in the percentage of nucleated cells in the sternal marrow.

5. A case of thyrotoxicosis following thyroid ingestion showed a marked decrease in the percentage of nucleated cells in the marrow when the thyroid was stopped. There was a less marked but definite decrease in cases where subtotal thyroidectomy was performed.

6. The hyperplasia found in the marrow of the hyperthyroid individuals was myeloid in character, and was not reflected in the peripheral blood.

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A QUANTITATIVE STUDY OF THE HEIGHT OF THYROID ACINAR CELLS IN NORMAL AND ABNORMAL THYROIDS.

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A study of the height of thyroid acinar cells and the attempted correlation thereof with any clinical thyroid disease is complicated by the multiplicity of factors known to affect the height of such cells, of which factors disease itself is only one. Indeed, disease is probably an effect of the other factors, at least in part. Presumably one factor that may be operative is the endocrine mediated through the thyrotropic hormone of the anterior pituitary. The potency of this hormonal effect has been more definitely confirmed

in recent years in experimental animals by observations such as those of Grant,[5] noting an increase in size of acinar cells during metamorphosis in amphibia, and those of Rawson and Starr,[9] definitely establishing a quantitative correlation of increased height of thyroid acinar cells with injection of thyrotropic hormone in guinea pigs. Also, it is well known that the heights of these cells tend to increase during menstruation and pregnancy, and as the result of cold. According to Aron and Dobrzaniecki,[3] while the cervical sympathetics exert no effect on cell size of themselves, their presence is necessary for the action of the pituitary. Flattening of the epithelium, together with increased fibrosis, is found in the thyroids of the aged.[6]

These effects on cell height and thyroid activity may be exerted unevenly, so that only a portion of the thyroid is active at one time. It would appear, according to Aron,[2] that some acini are actively secreting all the time but that the majority work in cycles. In pathologic thyroids this diversity of activity is especially evident, and indeed reaches its culmination, in nodular goiters. This cyclic variation in thyroid function and structure, as described by Marine and amplified by Rienhoff[10] helps to explain the wide variation in appearance of different sections, and the great difficulty in identifying a nodular goitre as toxic or non-toxic microscopically.

There have been many means devised to correlate the histologic appearance of sections with pathologic conditions and function. McFarland and Robson[7] found wide variations in acinar diameters in normal glands and no correlation between histologic appearance and age, sex or non-thyroid diseases. The mitotic index of Loeb is an accurate and objective index of thyroid activity, but is an exhausting and time-consuming method.[4] At present, dependence is usually placed on the impressions of the individual observer, a method neither open to objective analysis nor objectively accurate.

The purpose of these investigations was to see if any correlation of cell height and function existed in human thyroids, especially as it might aid in establishing an index whereby nodular goitres could be classified as to activity.

Method. The methods used in these experiments are modeled after those used by Rawson and Starr[9] in their experiments on guinea pigs. They correlated the average height and the mode of distribution of heights of 200 acinar cells as a measure of thyroid activity produced by the injection of thyrotropic hormone.

A micrometer scale fitted into the eyepiece of the microscope was used to measure the height of the cells, using an oil immersion objective. With the optical set-up used, and calibrated with a blood counting chamber, one unit is approximately equal to 1.4 μ. The diameter was measured in all cases at a point crosssing the nucleus and on a line along the radius of the acinus. The cells were measured in blocks of 100 from 100 separate acini chosen at random according to where the scale happened to fall as the slide was systematically moved across the stage. The only provisos were that the acini be open and that the cell boundaries be distinguishable.

Normal Thyroids. Thyroids were obtained from autopsies at Philadelphia General Hospital. The specimens were chosen at random, but an attempt was made to obtain specimens only from those in whom the cause of death had been comparatively acute and consequently not showing the effects of a long siege of illness. Because of the inadequate number of cases no attempt was made to classify the causes of death or attempt to correlate them with the results obtained. On microscopic section all glands appeared normal. In all, 40 normal thyroids were examined, of which 21 were from males between the ages of 20 and 50 and 19 from females between the ages of 20 and 70 (most between 20 and 50). No attempt was made to correlate our findings with age because the number is wholly inadequate.

First, 5 entire thyroids were obtained from male patients and 6 sections equally spaced obtained from each. Two sets of 100 cells were measured from each section of 2 of the glands and from 4 of the 6 sections of a third, and 1 set of 100 cells was measured from each of the other sections. It was found that nothing was to be gained by measuring more than 100 cells from any one of these sections (see Results).

In the remaining 35 glands, 100 cells from each were measured. Thus a total of 40 normal glands was examined, a number sufficiently large to apply statistical methods and obtain reasonably valid results. Statistics for these observations—mean, standard error, and range for each slide and for the group as a whole—were calculated. The first 21 of these normal glands are from men, the last 19 from women. The two groups were compared statistically to determine whether there was any detectable difference between them.

Toxic Diffuse Goitres. In the same fashion as with the normal glands, 100 cells from sections of 25 cases of toxic diffuse goitre were examined. Similar statistics were calculated for this group and analyzed.

Nodular Goitres. Sections (through the nodules) of 51 surgical specimens of nodular goitres were examined. The slides of all, toxic and non-toxic, were assembled and measured without knowing the clinical diagnosis so as to make the examinations as objective as possible. Because of the variability of different parts of nodular goitres it was deemed advisable to change the technique of examination somewhat. However, the number of sections from any one case was definitely limited. The procedure adopted was to measure 100 cells from one section as above and calculate the statistics. Then, if more sections were available, 50 cells were measured from each of them and if there was any section where the mean of the 50 cells was significantly different from the figure for the original 100, 100 cells from that section were also measured.

The nodular goitres were divided into two groups of 26 and 25 cases respectively, toxic and non-toxic, according to clinical signs of toxicity and basal metabolic rate. The signs of toxicity were given more weight and whenever present the gland was put in the toxic group. Three glands from cases showing definite elevation of BMR (basal metabolic rate) but no clinical signs of toxicity were not considered in the analysis in order to avoid possible borderline cases.

Results. *Normal Thyroids.* Despite the fact that there is a wide variation in measurements of individual cells, there was no difference greater than 0.2 units between the means of any two groups of 100 cells from any one gland of the 5 analyzed in multiple sections. The figure of 0.2 was obtained in only two instances. Inasmuch as twice the standard error is also 0.2 for these means, that difference does not constitute a significant one. Since the standard error of the mean is a measure of the variability of the

material studied, there is no indication that the normal thyroids studied are anything but uniform throughout as regards these means and there is presumably no error entailed in taking 100 cells from any one section of the gland.

When we consider the measurements of all 40 normal glands, we find that the range of means is well limited—from 3.6 to 4.9 units. The cell heights of 25 normal glands (21 males and 4 females) are plotted in Chart 1. The peaks of these curves, which represent the modes, are not so important as their general contour and position which represent graphically their means and distribution. The average of these means is 4.3 units and has a standard deviation of 0.3 units. The standard deviation referred to is a statistic designed to serve as a measure of variability of the whole group of means.

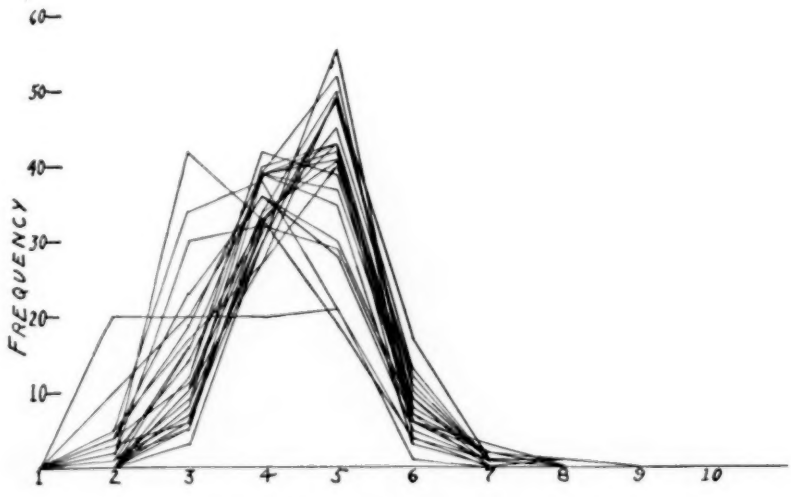

CHART 1.—Normal thyroids. Cell heights in units (1 unit = 1.4μ.)

So, if the normal glands measured be an adequate and representative sample of normal glands, the indication is that the mean cell height of 100 cells from these or any other normal glands will lie between that average of means plus and minus twice the standard deviation, or between 3.7 and 4.9 units, in over 95% of cases.

When we consider the normal glands in two groups, the first 21 comprising those from males and the remaining 19 from females, we find no statistical difference between the two groups. The average of means for the former is 4.4 units and for the latter is 4.3 units. The difference of 0.1 unit falls far short of statistical significance when we use either the standard error or Student's "t" criterion. (Student's "t" criterion is a measure of variability of means especially designed to be applicable in cases like these where the number

of cases is a little too small for the standard error to be rigidly applicable.)

Toxic Diffuse Goitres. Microscopically, sections of toxic diffuse goitres show cells that are obviously larger than those in normal glands. Even after iodine administration the difference is usually still distinguishable. Thus 22 of the 25 glands considered have mean cell heights outside the upper limit established for normal glands, with the average of all 25 means equal to 5.4 units. From the standpoint of variability these glands are intermediate between the normal glands already discussed and the nodular glands to be considered later. Of the 25, 19 have means between 5.0 and 6.0 units, but the others vary considerably. Statistically, the standard deviation of the average of these means is 0.6 units and so the limits

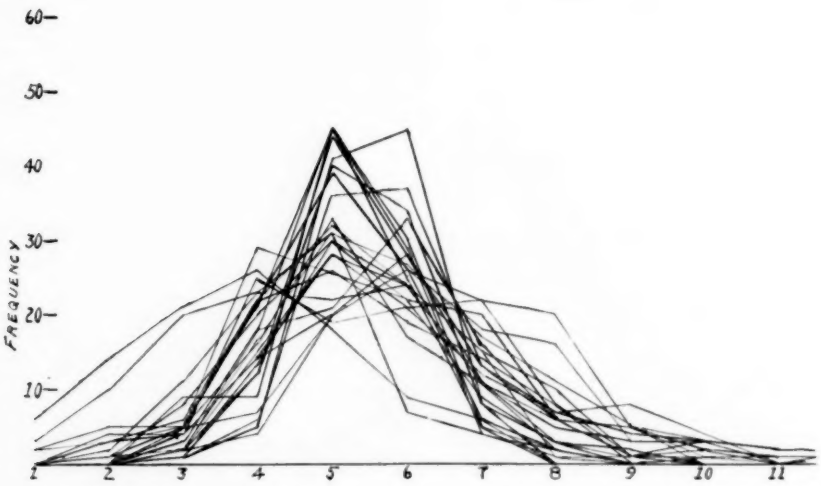

CHART 2.—Toxic diffuse goitres. Cell heights in units (1 unit = 1.4μ.)

are from 4.2 to 6.6 units for the means of cell heights of toxic diffuse goitres. Such limits are obviously too wide to be of any value. However, there is a very pronounced tendency for such means to be outside the upper limit for normal glands. (See Chart 2.)

Nodular Goitres. The variegated microscopic appearance of nodular goitres well described by Marine[8] is amply attested by the wide variation of cell heights and by the occasional variation in the mean heights of cells from different sections of the same gland (see Charts 3 and 4).

There is no sharp differentiation between the two groups of nodular goitres considered. In general, the means of cell heights from the group of toxic goitres are higher, but there is a wide overlapping in individual cases. Thus, mean heights from at least one

section of 18 of the 26 glands in the toxic group are over 5 units, while the same is true for only 11 in the other group. That propor-

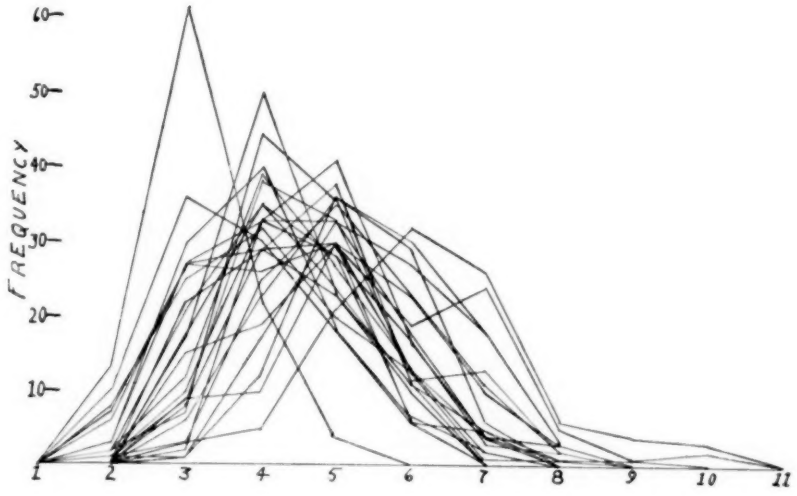

CHART 3.—Nodular goitres—Non-toxic. Cell heights in units (1 unit = 1.4μ.)

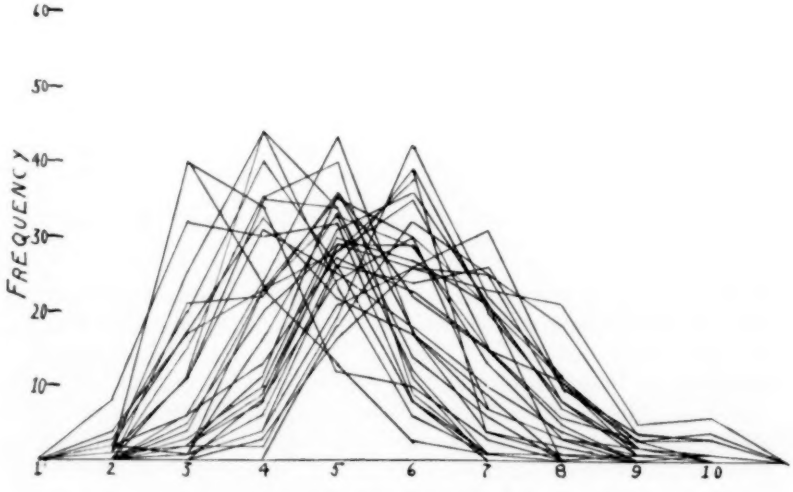

CHART 4.—Nodular goitres—Toxic. Cell heights in units (1 unit = 1.4μ.)

tion is 69% *versus* 44% outside the upper normal limit. Perhaps this definite tendency would be more apparent in glands where iodine had not been given preoperatively.

Of more value, however, is the fact that 10 of the 26 toxic glands have sections with a mean height of 5.8 units or more—a total of 38.5%. In the non-toxic group there is no mean height greater than 5.7 units. This seems to be a definite difference in the two groups and, although nothing is absolutely proven, it is probable that given a nodular goitre with cells of a mean height of about 6 units or greater, the goitre is toxic. Having an indication of toxicity in even a small percentage of cases is a distinct advantage because the difficulty of recognizing the difference by a subjective examination is such that many pathologists will not attempt the diagnosis. In this regard we were interested to note that of three glands with no associated clinical toxicity but consistently high elevation of

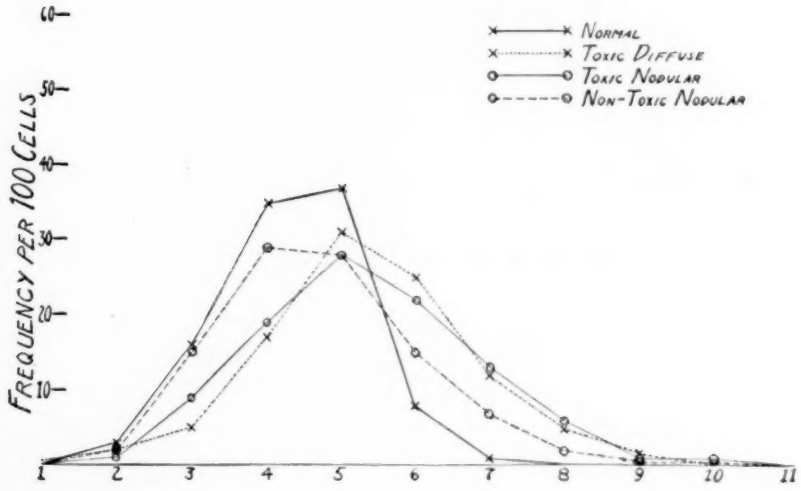

Chart 5.—Normal and pathological thyroids composite curves. Cell heights in units (1 unit = 1.4 μ.)

B.M.R. (not included in the series because of doubt as to classification), two had mean cell heights well above 6 units, which, we would be inclined to believe, indicated at least an impending toxicity manifested now not subjectively but only by increase in B.M.R.

In regard to possible effects of menstrual phase on the pathologic specimens, it is worth noting that goitre operations are preferably performed at a time when the patient is most stable—it is therefore assumed that menstrual effects do not play a rôle.

The problem of studying our human thyroids was complicated by several factors. First, those contributing normal glands were obviously not under the standard conditions of guinea pigs. Second, we have studied thyroids complicated by disease conditions definitely

more complex than a simple excess of hormone, and in all probability involving other factors beside pituitary dysfunction. And third, the disease pictures seen were from pathologic specimens at the University Hospital, where iodine is given routinely preoperatively to cases with toxic goitres and in some cases, when indicated, to those with non-toxic nodular goitres, tending to decrease the height of the glandular cells considerably.

Even under these circumstances, however, differences do appear between our groups of glands and are shown in the composite curves of Chart 5, wherein are plotted the average values of cell heights of each group. The normal curve is high and narrow and to the left of the others showing a comparatively narrow range of heights of relatively low cells. The curve of the toxic diffuse goitres is shifted to the right but is also fairly narrow, indicating greater cell height in a somewhat limited range. The curves of the nodular goitres are broad and low, showing the great variability of cell height, and the generally higher cell height of the toxic goitres is indicated by the shift of that curve to the right.

Summary and Conclusions. 1. Single and multiple sections of 116 normal and pathologic thyroid glands were examined microscopically and blocks of 100 acinar cells measured by means of a micrometer scale in the eyepiece. The method is easy, and with practice is comparable to a differential cell count.

2. There was found an almost perfect agreement of means of cell heights in different sections of 5 normal glands.

3. Means from 40 normal glands varied within a narrow range. The range of statistical significance is 3.7 to 4.9 of our units.

4. Twenty-five toxic diffuse goitres were examined and 22 of them had mean cell heights outside the upper limit of normal. The means of these glands were more variable than the normal but there was definite tendency for a fairly close grouping between about 5 and 6 units.

5. Fifty-one nodular goitres were examined and divided into toxic and non-toxic groups.

In general, there was an overlapping of the mean heights of the two groups although those of the former tended to be greater.

Of differential importance, however, was the fact that 38.5% of the former group had mean heights of 5.8 units or greater—a figure higher than any obtained in the latter group.

6. The indication follows that if these results be confirmed, the method used may serve as a useful index for distinguishing toxic nodular goitres, and perhaps for recognizing structural concomitants of thyroid hyperfunction as well.

The author wishes to acknowledge gratefully the help of Dr. I. T. Zeckwer in suggesting the problem, helping obtain material, and offering constructive criticism.

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INFLUENCE OF ESTROGEN ON THE INSULIN REQUIREMENT OF THE DIABETIC.

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The first attempt to reduce glycosuria by the use of estrogenic substance was that of Barnes, Regan and Nelson,[1] who reported their results in 1933. Because of the evidence indicating that the administration of the estrogenic hormone may suppress the sex principle of the pituitary, these investigators were interested in determining whether the diabetic principle of the pituitary might likewise be suppressed. Using pancreatectomized dogs they were able to control glycosuria with estrogenic substance. They attributed their results to complete suppression of the pituitary.

A year later Nelson and Overholser[3] reported on the effect of estrin upon experimental pancreatic diabetes in the monkey. In their animals, alternate periods of injection and withdrawal of estrin resulted in corresponding fluctuations in the urinary sugar. These investigators also attributed their results to suppression of the pituitary.

Mazer[2] and his coworkers, in 1935, reported that while treating menopausal patients with large doses of female sex hormone, the 3 diabetics in their series could be maintained on a reduced insulin dose. Withdrawal of estrogen was associated with a return to the original insulin requirement. A study of the blood sugar levels of normal women under similar circumstances indicated that in these patients the blood sugar level was unaffected.

The only clinical cases reported were those of Patton,[4] who, in 1936, demonstrated successful results with the clinical use of large doses of female sex hormone in 3 cases. One of these is worthy of note in that recurrent coma during the menstrual periods was prevented by the use of large doses of estrogenic hormone.

In our investigation we were interested in studying the effect of intramuscularly administered estrogen on the insulin requirement

of a series of diabetic women. Nine cases were taken from the Metabolic Service of Sea View Hospital. They were chosen without regard for any indication for estrogen therapy. The menstrual history of these patients, however, is of interest in that only 1 member of the group was menstruating regularly. Three had periods of amenorrhea of several months' duration and 5 had passed through the menopause.

TABLE 1.—VARIATIONS IN INSULIN DOSAGE WITH ESTROGEN (OBSERVATIONS ON 9 CASES FROM THE METABOLIC SERVICE OF SEA VIEW HOSPITAL, OCTOBER, 1938, TO OCTOBER, 1939).

Date of observation.	Units of insulin.									
	Average.	AN.	CH.	CA.	RO.	AR.	BI.	PE.	RA.	WI.
1938.					Before Estrogen.					
10/1	63	35	45	..	50	90	70	112	75	30
10/15	68	35	45	..	45	80	90	130	75	40
10/29	76	35	40	..	40	80	110	150	75	80
11/11	76	40	40	..	40	90	90	150	75	80
11/25	78	40	40	*	40	90	85	173	75	80
12/9	76	40	40	65	40	90	85	170	75	80
12/23	76	40	40	60	40	90	85	170	75	80
1939.										
1/8	76	42	40	60	40	90	85	173	75	80
1/22	75	42	40	60	40	90	85	180	75	80
2/7	84	42	40	70	40	110	90	180	80	80
					During Estrogen.					
2/15	80	42	40	70	40	110	90	180	80	70
2/22	80	38	40	70	40	100	87	180	75	70
3/1	75	30	35	70	40	100	85	170	70	70
3/8	66	25	30	65	40	90	73	160	65	65
3/22	61	15	20	60	35	85	70	145	60	55
4/10	46	5	10	50	20	90	50	125	45	25
4/23	43	0	10	45	15	90	43	115	45	20
5/3	47	5	10	45	15	90	75	115	45	20
5/19	49	12	20	55	15	90	75	115	45	15
6/3	48	15	20	55	15	90	70	115	50	5
6/21	51	15	20	65	25	90	70	120	55	0
7/9	54	15	20	80	30	90	70	120	60	0
					After Estrogen.					
7/16	56	15	20	80	30	90	70	120	80	0
7/23	57	15	20	80	30	90	70	130	80	0
8/1	58	15	20	80	30	90	80	120	80	0
8/8	57	15	20	80	30	90	80	120	80	0
8/20	53	15	20	80	25	90	80	95	75	5
8/30	54	15	5	75	35	90	85	100	70	0
9/8	53	25	10	65	35	75	85	120	60	0
9/22	62	25	20	50	40	115	95	150	65	0
10/1	62	25	22	60	40	125	95	130	65	0
10/12	63	30	22	65	40	130	65	145	70	0

* Prior to date of initial observation.
"Units of insulin" represents daily requirement of regular, protamine zinc or both.

The method of administration was that generally used in the treatment of the menopausal syndrome and therefore differed from that used in previous experiments where daily injections were usually given. Our patients received an intramuscular injection of 10,000 International Units of estrogenic hormone twice a week.

To determine the variations in the average insulin requirement before, during and after the administration of estrogen, the patients were followed for a period of 1 year; 4 months before estrogen, 5 months during its administration and 3 months after it had been discontinued.

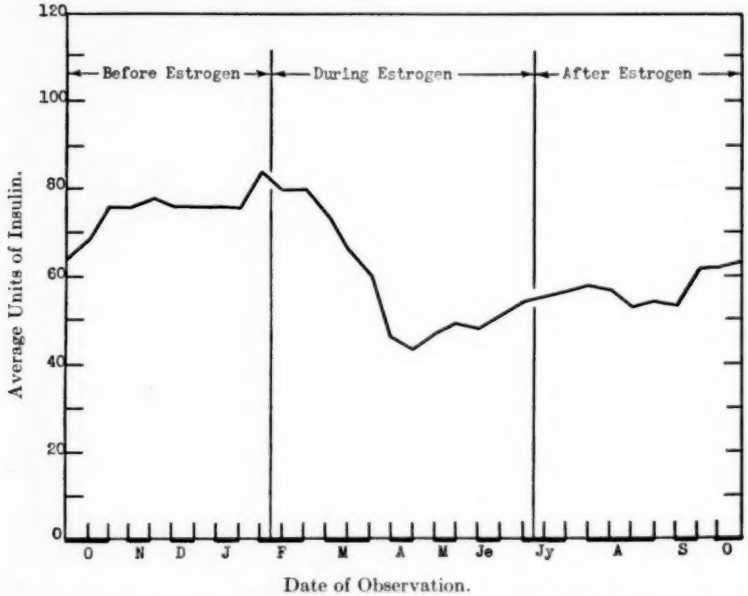

CHART 1.—Variations in average insulin dosage (regular and PZI) with estrogen.

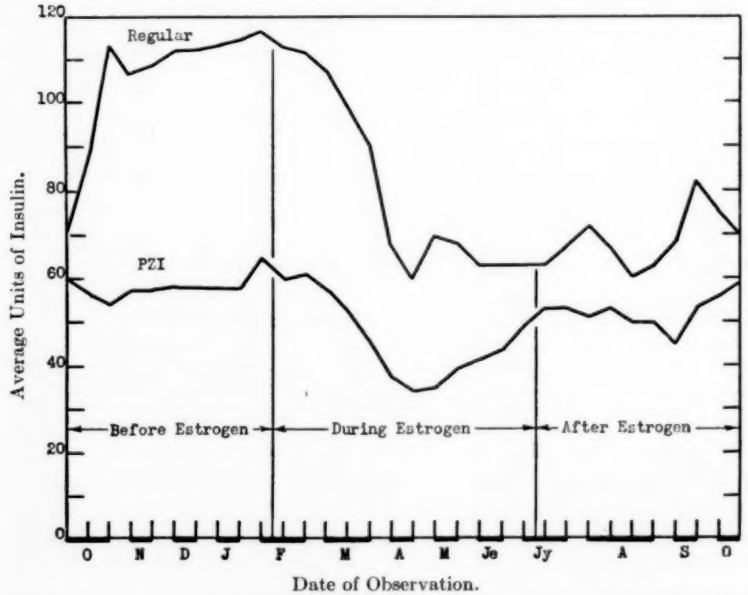

CHART 2.—Variations in insulin dosage with estrogen. Comparison of regular insulin with protamine zinc insulin.

During the first 4 months we attempted to reduce the individual insulin dose to the lowest level compatible with proper control. We took as our standards of control, a normal fasting blood sugar, a glycosuria of less than 1% with a total loss of less than 20 gm. of sugar in the 24-hour urine. At no time during this 4-month period of observation was there any indication that the average daily insulin requirement could be materially reduced. Instead it rose from 63 to 84 units daily (Table 1). Soon after estrogen was begun,

TABLE 2.—VARIATIONS IN INSULIN DOSAGE WITH ESTROGEN (OBSERVATIONS ON 9 CASES FROM THE METABOLIC SERVICE OF SEA VIEW HOSPITAL, OCTOBER, 1938, TO OCTOBER, 1939).

Date of observation.	Comparison of regular insulin with protamine zinc insulin, units of insulin.										
	Regular.				Protamine zinc.						
	WI.	PE.	BI.	Av.	Av.	RA.	AR.	RO.	CA.	CH.	AN.
1938.					Before Estrogen.						
10/1	32	112	70	71	59	75	90	50	..	45	35
10/15	40	130	90	87	56	75	80	45	..	45	35
10/29	80	150	110	113	54	75	80	40	..	40	35
11/11	80	150	90	107	57	75	90	40	..	40	40
11/25	80	173	85	109	57	75	90	40	*	40	40
12/9	80	170	85	112	58	75	90	40	65	40	40
12/23	80	170	85	112	58	75	90	40	60	40	40
1939.											
1/8	80	173	85	113	58	75	90	40	60	40	42
1/22	80	180	85	115	58	75	90	40	60	40	42
2/7	80	180	90	117	64	80	110	40	70	40	42
					During Estrogen.						
2/15	70	180	90	113	60	80	110	40	70	40	42
2/22	70	180	87	112	61	75	100	40	70	40	38
3/1	70	170	85	108	58	70	100	40	70	35	30
3/8	65	160	73	99	53	65	90	40	65	30	25
3/22	55	145	70	90	46	60	85	34	60	20	15
4/10	25	125	50	67	37	45	90	20	50	10	5
4/23	20	115	45	60	34	45	90	15	45	10	0
5/3	20	115	75	70	35	45	90	15	45	10	5
5/19	15	115	75	68	39	45	90	15	55	20	12
6/3	5	115	70	63	41	50	90	15	55	20	15
6/21	0	120	70	63	43	55	90	25	55	20	15
7/9	0	120	70	63	49	60	90	30	80	20	15
					After Estrogen.						
7/16	0	120	70	63	53	80	90	30	80	20	15
7/23	0	130	70	67	53	80	90	30	80	20	15
8/1	0	135	80	72	51	70	90	30	80	20	15
8/8	0	120	80	67	53	80	90	30	80	20	15
8/20	5	95	80	60	50	75	90	25	80	10	15
8/30	0	100	85	62	50	70	90	35	75	5	25
9/8	0	120	85	68	45	60	75	35	65	10	25
9/22	0	150	95	82	53	65	115	40	50	20	25
10/1	0	130	95	75	56	65	125	40	60	22	25
10/12	0	145	65	70	59	70	130	40	65	22	30

* Prior to date of initial observation.

however, the average daily insulin requirement began to fall so that within a period of 10 weeks it dropped from 84 to 43 units daily, representing a saving of 47% (Table 1). During the second 10 weeks of the administration of estrogen the average daily insulin requirement rose to 54 units, an increase of 11 units, but still 33% less than before estrogen was begun (Table 1). At this time estrogen was discontinued. Nevertheless the patients were followed 3 months longer. During this period the average daily insulin requirement rose to 63 units (Table 1), an increase of only 9 units

and still 21 units less than the amount used immediately preceding the beginning of estrogen (Chart 1).

TABLE 3.—VARIATIONS IN INSULIN DOSAGE WITH ESTROGEN (OBSERVATIONS ON 9 CASES FROM THE METABOLIC SERVICE OF SEA VIEW HOSPITAL, OCTOBER, 1938, TO OCTOBER, 1939).

	Comparison of premenopausal and postmenopausal cases, units of insulin.										
	Premenopausal.					Postmenopausal.					
Date of observation.	AN.	CH.	CA.	RO.	Av.	Av.	AR.	BI.	PE.	RA.	WI.
1938.					Before Estrogen.						
10/1	35	45	..	50	43	75	90	70	112	75	30
10/15	35	45	..	45	42	83	80	90	130	75	40
10/29	35	40	..	40	38	99	80	110	150	75	80
11/11	40	40	..	40	40	97	90	90	150	75	80
11/25	40	40	*	40	40	101	90	85	173	75	80
12/9	40	40	65	40	46	100	90	85	170	75	80
12/23	40	40	60	40	45	100	90	85	170	75	80
1939.											
1/8	42	40	60	40	46	101	90	85	173	75	80
1/22	42	40	60	40	46	102	90	85	180	75	80
2/7	42	40	70	40	48	108	110	90	180	80	80
					During Estrogen.						
2/15	42	40	70	40	48	106	110	90	180	80	70
2/22	38	40	70	40	47	102	100	87	180	75	70
3/1	30	35	70	40	44	99	100	85	170	70	70
3/8	25	30	65	40	40	90	90	73	160	65	65
3/22	15	20	60	35	33	83	85	70	145	60	55
4/10	5	10	50	20	21	67	90	50	127	45	25
4/23	0	10	45	15	18	63	90	42	115	45	20
5/3	5	10	45	15	19	69	90	75	115	45	20
5/19	12	20	55	15	26	68	90	75	115	45	15
6/3	15	20	55	15	21	66	90	70	115	55	0
6/21	15	20	65	25	31	67	90	70	120	55	0
7/9	15	20	80	30	36	68	90	70	120	60	0
					After Estrogen.						
7/16	15	20	80	30	36	72	90	70	120	80	0
7/23	15	20	80	30	36	74	90	70	130	80	0
8/1	15	20	80	30	36	74	90	80	120	80	0
8/8	15	20	80	30	36	74	90	80	120	80	0
8/20	15	20	80	25	35	69	90	80	95	75	5
8/30	15	5	75	35	33	69	90	85	100	70	0
9/8	25	10	65	35	34	68	75	85	120	60	0
9/22	25	20	50	40	34	85	115	95	150	65	0
10/1	25	22	60	40	37	83	125	95	130	65	0
10/12	30	22	65	40	39	82	130	65	145	70	0

* Prior to date of initial observation.
"Units of insulin" represents daily requirement of regular, protamine zinc or both.

The type of insulin administered did not influence the results obtained. Table 2 indicates that during the first 11 weeks of the administration of estrogen there was a drop of 47%, from 117 to 60 units daily, in the average insulin requirement of the patients receiving regular insulin. During the same time the patients on protamine zinc insulin showed a similar saving represented by a decrease in the average daily insulin requirement from 64 to 34 units. The percentage of insulin saved in the two groups is identical, although the absolute drop in the insulin requirement is greater in the group which received regular insulin (Chart 2).

If the cases are separated into those who have passed the menopause and those who are premenopausal, it becomes evident that the decrease in the insulin requirement associated with the administration of estrogen is more pronounced in the premenopausal group.

In the younger group, the drop from an average of 48 units daily to an average of 18 units represents an insulin saving of 63%. In the older group, on the other hand, the initial average requirement of 108 units daily fell with estrogen to 63 units, a saving of 42% (Table 3). When estrogen was discontinued the younger patients were receiving an average of 36 units of insulin daily. In the succeeding 3 months their requirement rose to 39 units daily, an increase of less than 1%. In the older group, however, there was a rise in the average insulin requirement of 20% for the same period of time, from 68 to 82 units daily (Table 3). The data therefore

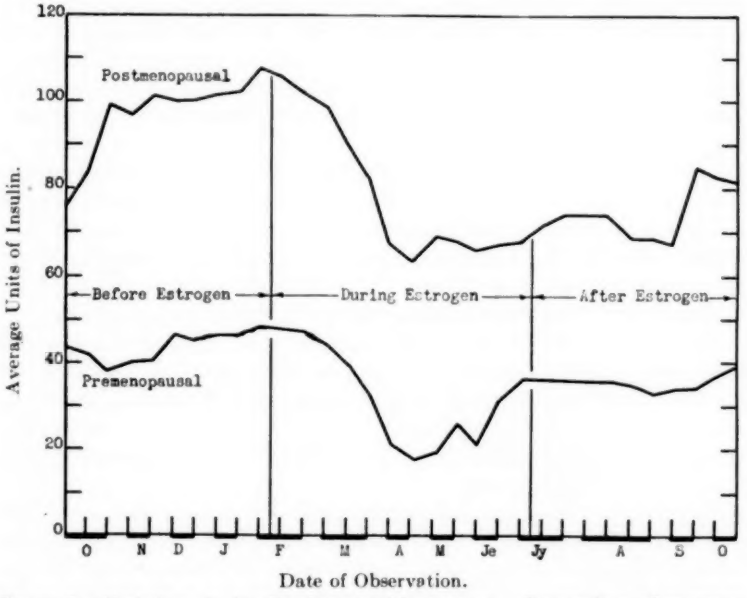

CHART 3.—Variations in insulin dosage with estrogen. Comparison of premenopausal with postmenopausal cases.

indicate that the administration of estrogen was associated with a greater and more permanent reduction in the insulin requirement in the premenopausal than in the postmenopausal group (Chart 3).

Untoward Effects. During the administration of estrogen we encountered several untoward effects. Some of our patients complained of severe headache starting soon after the injection and lasting 24 to 36 hours. One patient had painful, tender breasts which subsided 4 days after estrogen was discontinued. Another of our patients developed a fibroid uterus. Whether this was a coincidental development or in some way associated with the administration of estrogen is difficult to determine.

Discussion. In interpreting our results it is significant that all our patients, regardless of age and endocrine constitution, received the same amount of estrogenic hormone at the same time interval. A similar amount of estrogen, however, will not produce a like effect in different individuals. The results might have been more spectacular had we been able to vary the amount of estrogen administered to each patient. Because of the obvious difficulties such a task entails in a study of this type a common dose was administered to all our patients.

The mode of action of estrogen in depressing the glycosuria of the diabetic is difficult to explain. Barnes and his coworkers assumed that estrogen caused a complete suppression of the pituitary and warned that such suppression might be dangerous because it might involve atrophic changes in other organs, especially the adrenal cortex. Nelson and Overholser suggested that the effect may be due to an inhibition of the pituitary diabetogenic hormone. This explanation seems plausible, especially since the pituitary is the only gland known to have pancreatotropic and gonadotropic hormones, both of which are most likely derived from the basophil cells. Furthermore, it is significant that diabetes is often aggravated during the periods of gonadal stress, as at puberty, menstruation, pregnancy and the menopause. The *modus operandi* in the gonado-pituitary-pancreatic relationship, however, is still indefinite.

It is of interest that our postmenopausal patients did not respond so well as our younger group. In the older patients the function of all anterior pituitary cells is decreased. If the action of estrogen on carbohydrate metabolism is by way of the pituitary, as we have reason to assume, we can readily understand the better results in the premenopausal group.

Conclusions. 1. The administration of 10,000 International Units of estrogenic hormone twice a week to a series of 9 diabetic women was associated with a diminution in their insulin requirement.

2. The average daily insulin requirement did not return to the "pre-estrogen" level even after estrogen had been discontinued for a period of 3 months.

3. The type of insulin used in the control of the diabetes had no influence on the results obtained.

4. In the premenopausal group the diminution in the insulin requirement associated with the administration of estrogen was greater and more sustained than in the postmenopausal group.

We are indebted to Roche-Organon Company and to E. R. Squibb for our supply of estrogenic hormone.

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THE CALCIUM AND PHOSPHORUS IN THE CEREBROSPINAL FLUID IN DIABETES INSIPIDUS.

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Studying the blood in a group of 26 patients with diabetes insipidus, I[1] found a slight but definite increase in the serum calcium in some of the cases. Because of the interest in the relation of the calcium content of the spinal fluid and blood serum and because of the similarity between the polyuria of diabetes insipidus and the polyuria which occurs in certain cases of hyperparathyroidism, the concentration of the calcium and phosphorus in the cerebrospinal fluid was studied in 10 patients with diabetes insipidus and compared with those in the blood serum. In addition, the chloride and protein contents were determined.

The general impression is that the concentration of the calcium in the cerebrospinal fluid is remarkably constant in health and disease. The calcium normally varies from 4.5 to 5.5 mg., an average of 5 mg. per 100 cc. of cerebrospinal fluid[9] and it represents 45 to 50% of the blood serum calcium. The phosphorus content varies from 1 to 1.5 mg. per 100 cc. and usually amounts to 38% of the serum phosphorus. McLean and Hastings[7] have shown that the calcium in the serum is composed of a diffusible fraction which represents about 50% of the total calcium and passes through a membrane impermeable to protein, and a non-diffusible fraction which amounts to about 50% of the total calcium and remains with the protein in the serum. They found that the diffusible calcium of the serum is all or nearly all ionized and that consequently the diffusible calcium is a measure of Ca^{++} concentrations. They made a number of observations on human cerebrospinal fluid and found that the spinal fluid contains about 5 mg. calcium per 100 cc. fluid which is all diffusible and all but an insignificant amount is in the form of Ca^{++}. They believed that the spinal fluid calcium concentration represents the diffusible or ionized portion of the serum calcium. However, there are certain diseases in which there has been reported an increase in the calcium of the cerebrospinal fluid, particularly the meningitic or other fluids with a high protein content. Cantarow[2] found the calcium content of 4 to 7.5 mg. per 100 cc. of cerebrospinal fluid in patients with inflammatory disease of the meninges and brain. Merritt and Bauer[8] also found an increase in the spinal fluid calcium in meningitis. Cohn, Kaplan, and Levinson[4] obtained similar results in such cases and also in cases with hydrocephalus and brain tumor, in addition to an increase in the spinal fluid phosphorus. Gregory and Andersch[6] found the spinal fluid calcium to be as high as 6.4 mg. per 100 cc. in 1 of 2

cases of hyperparathyroidism. This paper presents the results of the calcium, phosphorus, chloride and protein concentrations in the spinal fluid and in the blood serum of 10 patients with diabetes insipidus.

Plan of Investigation. The spinal fluid was obtained by lumbar puncture from 10 patients with diabetes insipidus whose ages ranged from 16 to 67 years. The criteria for the diagnosis of diabetes insipidus were a persistent daily fluid intake and output of about 8 to 14 liters for years, which were reduced to normal following the intranasal or intramuscular administration of pituitary extract (pituitrin).

There were 8 males and 2 females. The disease was idiopathic in origin in 8 cases and postencephalitic in 2, both males. The calcium was determined by the Clark and Collip modification of the Kramer-Tisdall method.[3] The phosphorus was determined by the method of Fiske and Subbarow.[5]

Having demonstrated that the distribution of calcium between Ca^{++} and Ca bound to protein depends on the total protein, McLean and Hastings[7] showed how to calculate the Ca^{++} concentrations from values for total calcium and total protein in serum and based on these values they made a Cartesian nomogram from which the Ca^{++} concentrations may be determined.

In this investigation the Ca^{++} concentration in the blood serum of the 10 patients with diabetes insipidus was calculated according to their nomogram and the results compared with the calcium determinations in the spinal fluid and the differences noted. Control tests were made in 10 patients with syphilis, in whom diagnostic lumbar punctures were performed.

TABLE 1.—COMPARISON OF CONCENTRATIONS OF Ca^{++} IN SERUM AND TOTAL CALCIUM IN SPINAL FLUID IN CONTROL PATIENTS.*†

	Serum.			Spinal fluid, total Ca.	Spinal fluid Ca minus serum Ca^{++}.
Case.	Total Ca.	Total protein.	Calculated Ca^{++}.		
1	2.94	66	1.38	1.38	0
2	2.96	70	1.34	1.00	−0.34
3	2.90	70	1.32	1.15	−0.17
4	2.69	72	1.24	1.20	−0.04
5	2.63	68	1.16	1.32	+0.16
6	2.63	70	1.13	1.28	+0.15
7	2.90	75	1.27	1.40	+0.13
8	2.58	71	1.11	1.28	+0.17
9	2.74	69	1.17	1.35	+0.18
10	2.91	71	1.31	1.40	+0.09
Average	2.79	70	1.24	1.27	+0.03

* The calcium results are recorded in mM (millimol) per liter of water and the total protein in gm. per liter of serum.

† All the control patients were syphilitics who had been under anti-luetic treatment in the past. There was no other diagnosis.

Results. The results in the spinal fluid compared with those in the serum are given in the accompanying tables. In the control tests the calcium concentration of the spinal fluid ranged from 3.9 to 5.6 mg. per 100 cc. or from 1 to 1.4 mM per liter of spinal fluid, the average being 1.27 mM per liter. In the serum, the total calcium varied from 9.5 to 11 mg. per 100 cc. or from 2.58 to 2.96 mM per

liter of water, the average being 2.79 mM. The Ca^{++} concentrations of serum ranged from 1.11 to 1.38 mM per liter of water, the average being 1.24 mM. The total calcium in the spinal fluid was nearly the same as the Ca^{++} concentration of the serum, the average difference being +.03 mM per liter which corresponds with the results obtained by McLean and Hastings. In 4 cases the difference ranged from 0 to −.34 mM per liter water and in 6 cases it varied from +.03 to +.18 mM per liter.

In the patients with diabetes insipidus the results were somewhat different. The total calcium in the spinal fluid ranged from 4.2 to 7.4 mg. per 100 cc. or from 1.05 to 1.85 mM per liter, the average being 1.51 mM. In 5 of these patients, the calcium ranged from 5.8 to 7.4 mg. per 100 cc. spinal fluid or from 1.45 to 1.85 mM per liter and in one of these patients whose spinal fluid calcium was 7.4 mg. a repeat spinal fluid in 3 months gave the same result.

TABLE 2.—COMPARISON OF CONCENTRATIONS OF Ca^{++} IN SERUM AND TOTAL CALCIUM IN SPINAL FLUID IN PATIENTS WITH DIABETES INSIPIDUS.*

Case.	Serum.			Spinal fluid, total Ca.	Spinal fluid Ca minus serum Ca^{++}
	Total Ca.	Total protein.	Calculated Ca^{++}.		
1	2.76	59	1.30	1.80	+0.50
2	2.91	71	1.31	1.50	+0.19
3	3.13	72	1.36	1.45	+0.09
4	2.26	71	0.90	1.05	+0.15
5	2.71	77	1.07	1.70	+0.63
6	2.69	71	1.13	1.38	+0.25
7	2.75	72	1.13	1.40	+0.27
8	2.68	66	1.19	1.33	+0.14
9	2.95	61	1.43	1.30	−0.13
10	2.98	61	1.44	1.85	+0.41
10	3.14	60	1.52	1.85	+0.33
Average	2.81	68	1.25	1.51	+0.26

* The calcium results are recorded in mM (millimol) per liter of water and the total protein in gm. per liter of serum.

In the serum, the total calcium ranged from 8.4 to 11.8 mg. per 100 cc. or from 2.26 to 3.14 mM per liter of water, the average being 2.81 mM. The serum Ca^{++} values were from .90 to 1.52 mM per liter of water, the average being 1.25 mM. The total calcium of the spinal fluid was appreciably greater than the Ca^{++} concentration in the serum in a number of these cases, the average difference being +.26 mM per liter of water as compared with +.03 mM in the control tests. In only one of these cases was the difference on the minus side. In the remaining cases, 4 had a difference of +.09 to +.19 mM per liter of water and 6 had a difference of +.25 to +.63 mM per liter. The ratio of the spinal fluid calcium to the serum calcium ranged from 47 to 69%.

The phosphorus content of the spinal fluid and serum was within the normal limits and the values were much the same as in the control tests.

An elevated total protein of from 64 to 150 mg. per 100 cc. spinal fluid appeared in 3 cases, 2 of which were associated with post-encephalitic Parkinson's syndrome. The serum protein determinations were normal.

TABLE 3.—COMPARISON OF FINDINGS IN SPINAL FLUID AND SERUM IN PATIENTS WITH DIABETES INSIPIDUS AND IN CONTROL PATIENTS.*

		Diabetes insipidus.				Control tests.		
Case.		Total Ca.	P.	Cl.	Total protein.	Total Ca.	P.	Total protein.
1	CSF	7.2	1.6	448	90.0	5.5	2.4	35.0
	Serum	10.4	5.0	318	5.9	11.0	4.1	6.6
2	CSF	6.0	1.2	461	64.0	3.9	2.1	30.0
	Serum	10.8	3.8	394	7.1	11.0	...	7.0
3	CSF	5.8	1.6	442	150.0	4.6	1.9	40.0
	Serum	11.6	4.4	335	7.2	10.7	...	7.0
4	CSF	4.2	1.0	467	30.0	4.7	1.7	5.0
	Serum	8.4	3.2	412	7.1	10.0	3.7	7.2
5	CSF	6.8	1.8	...	18.0	5.3	1.8	80.0
	Serum	10.0	3.6	...	7.7	9.8	...	6.8
6	CSF	5.5	...	...	...	5.1	1.9	35.0
	Serum	10.0	3.7	421	7.1	9.8	3.5	7.0
7	CSF	5.6	1.6	404	15.0	5.6	1.7	35.0
	Serum	10.2	3.9	375	7.2	10.7	2.7	7.5
8	CSF	5.3	2.7	382	37.0	5.1	1.7	20.0
	Serum	10.0	4.1	339	6.6	9.5	3.6	7.1
9	CSF	5.2	1.2	492	30.0	5.4	1.6	20.0
	Serum	11.1	3.8	314	6.1	10.2	3.8	6.9
10	CSF	7.4	1.6	451	41.0	5.6	1.6	30.0
	Serum	11.2	4.0	357	6.1	10.8	4.3	7.1
10	CSF	7.4	...	448	13.0			
	Serum	11.8	2.6	346	6.0			

* The results are expressed in mg. per 100 cc. except the serum protein, which is recorded in gm. per 100 cc. CSF indicates cerebrospinal fluid; P, phosphorus, and Cl, chloride.

The chloride concentration in the spinal fluid varied from 382 to 492 mg. per 100 cc. in these cases, the normal being approximately 440 mg. The chloride in the blood was within the normal range.

Comment. The cerebrospinal fluid has been of special interest because it has been regarded by certain investigators as normally a protein-free filtrate of the blood plasma and that its calcium content represents the diffusible or ionized calcium of the blood or approximately one-half of the total blood calcium concentration. Furthermore, the calcium content of the blood and spinal fluid is maintained at a markedly constant level. The results of this investigation indicate that there is a disturbance in the calcium level in some cases with diabetes insipidus. The average spinal fluid calcium concentration of 1.51 mM per liter was slightly greater than the average serum Ca^{++} values of 1.25 mM per liter of water as calculated from the total calcium and protein in the serum, according to the method described by McLean and Hastings. The average difference was +.26 mM per liter of water and the greatest difference was +.63 mM. This was in contrast to the control tests in which the average spinal fluid calcium was 1.27 mM per liter and the average serum Ca^{++} values of 1.24 mM per liter of water, an average difference of only

+.03 mM per liter of water. The cause for this is conjectural. The serum calcium varies with the serum protein. However, on this basis the spinal fluid calcium would not be increased because its protein content is so small that it would not appreciably alter its calcium concentrations. The fluids of the human body, including both protein-free and protein containing fluids, contain a small but appreciable amount of a bound but diffusible form of calcium. According to McLean and Hastings, this amount has been estimated to be of the order of magnitude of 0.15 mM per kilo of water. Possibly, in some of the cases of diabetes insipidus, the excess of the calcium of the spinal fluid over the calculated Ca^{++} concentrations of the serum may be due to an increase in the amount of bound but diffusible form of calcium. Whether or not such an explanation is correct must await direct determination of the Ca^{++} concentration of cerebrospinal fluid in comparable cases.

Summary. The concentrations of the calcium, phosphorus, chlorides and protein were studied in the spinal fluid and blood serum in 10 patients with diabetes insipidus.

The average concentration of the spinal fluid calcium was slightly greater than that of the Ca^{++} content of the serum (1.51 *vs.* 1.27 mM per liter) in these patients. A series of control patients showed no such difference between their serum Ca^{++} concentrations and cerebrospinal fluid calcium (1.27 *vs.* 1.24 mM per liter).

Analyses for inorganic phosphate, chloride and protein showed no significant differences.

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MAINTENANCE OF NITROGEN EQUILIBRIUM OF AMINO ACIDS ADMINISTERED PARENTERALLY.

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The maintenance of an adequate state of nutrition in surgical and medical patients where enforced fasting is unavoidable can be accomplished by parenteral feeding. Glucose infusions contribute

the necessary fluid requirement as well as calories for energy. However, the continuous destruction of body proteins necessitates the administration of protein for replacement. Proteins foreign to the human body and dissimilation products of protein as far down as peptones cannot be administered parenterally because they produce anaphylactic reactions. However, amino acids do not cause such reactions when given parenterally.

Abderhalden,[1] in 1909, was able to maintain nitrogen balance by feeding digested lean beef per rectum. McClendon, Cavett and Johnson[4] more recently were able to maintain nitrogen balance by the administration of amino acids per rectum. Elman[3] was the first to administer intravenous infusions of amino acids to man clinically. He demonstrated that nitrogen balance could be achieved by giving a mixture of amino acids[2] as the only source of nitrogen.

The following study was undertaken: 1, to find whether or not a mixture containing the essential amino acids might be administered to patients without untoward reactions; 2, to determine the best possible method of its administration; and 3, to determine whether patients given this mixture can convert the amino acids to body protein and can be kept in nitrogen balance.

The mixture of amino acids used in this investigation was prepared by one of us (M. S.). It is a hydrolysate of casein to which has been added 1.8% tryptophan and 1.5% cystine. It contains 1.0% nitrogen, 5.0% glucose, traces of calcium ions, 0.015% potassium chloride, and 0.7% sodium chloride. It is a clear, amber-colored fluid with a distinct smell of meat broth. Determinations show it to contain about 7 gm. amino acid nitrogen per 100 cc. It is sterile in aërobic and in anaërobic culture.

To determine whether this mixture could be given without anaphylactic reaction, 2 rabbits were injected intramuscularly with 5 cc. of the fluid three times at 6-day intervals and did not show any symptoms when reinjected intravenously after 21 days with 1 cc. and 2 cc. respectively. Thirty-five cubic centimeters of the fluid given subcutaneously to 2 rabbits was tolerated without reaction and was absorbed within 7 hours. Chemical tests for protein as well as polypeptides and albumoses were negative (sulphosalicylic acid, biuret reaction).

Subsequently, 13 patients were injected with the amino acids mixture. The patients selected had no diseases which might affect the protein metabolism, such as diseases of the liver or kidneys, diabetes mellitus, carcinoma, or infections. The patients chosen were orthopedic cases and postoperative cases in the Department of Gynecology. Five of these persons were under observation for 3 to 5 weeks and received daily subcutaneous infusions of 500 to 600 cc. of the amino acids mixture during the experimental period, each of which was 1 week long. Four patients received 1 to 300 cc. intravenously in amounts increasing daily. Four received daily intra-

venous injections of 500 to 750 cc. and were under observation for from 18 to 21 days. Thus, there were 5 patients who received the amino acids mixture subcutaneously and 8 who received it intravenously.

TABLE 1.—NITROGEN BALANCE FIGURES (DAILY AVERAGES) DURING PARENTERAL ADMINISTRATION OF AMINO ACIDS AND IN CONTROL PERIOD.

Case number.	Period.	Intake.					Output.		Amino acid-N utilized, gm.
		Protein, gm.	Protein nitrogen, gm.	Amino acid, cc.	Amino acid nitrogen, gm.	Total nitrogen, gm.	Total nitrogen, gm.	Amino acid nitrogen, gm.	
1	Control	60	9.6			9.6	8.20	2.49	
	Experimental	20	3.2	500	5.0	8.2	11.91	2.79	4.70
2	Control	80	12.8			12.8	7.61	1.50	
	Experimental	40	6.4	600	6.0	12.4	10.88	2.75	4.75
3	Control	60	9.6			9.6	5.10	1.45	
	Experimental	20	3.2	500	5.0	8.2	6.63	2.32	4.13
4	Control	70	11.2			11.2	7.26	1.70	
	Experimental	30	4.8	500	5.0	9.8	7.84	2.20	4.50
5	Control	65	10.4			10.4	9.30	1.55	
	Experimental	25	4.0	600	6.0	10.0	8.01	2.20	5.35
6	Control	70	11.2			11.20	7.33	1.80	
	Experimental	38	6.8	500	5.0	11.08	8.00	2.66	4.14
7	Control	65	10.40			10.40	7.60	1.28	
	Experimental	33	5.28	500	5.0	10.28	7.58	2.37	3.91

For 7 days before the experimental period the patients were given a high carbohydrate diet of 45 to 60 calories and 1.0 gm. of protein per kg. of body weight. The urines were collected throughout each 24-hour period. Determinations of the total nitrogen (by Nesslerization), urine amino acids (by the method of Folin as modified by Sahyun[5]), creatine and creatinine were made daily. The nitrogen contents of the stools were not determined. Since none of the patients had a diarrhea during the investigation, the nitrogen contents of the stools were considered to be 1.0 to 2.0 gm. per day. In the orthopedic patients studied, after 7 days of control period, part of the food protein was replaced by a corresponding amount of amino acids mixture for the following 7 days. The same urine studies were made daily. Thereafter a second control period similar to the first followed.

The injections were uniformly well tolerated. The mixture was diluted with an equal amount of sterile water which had been warmed to body temperature. The time required for the injection of 1000 cc. of fluid was gauged to the comfort of the patient; it usually was 4 to 5 hours. The resorption time was found to be up to 12 hours.

Tables 1 and 2 show the results obtained with 9 patients. The figures represent averages of the daily determinations for each patient during each period.

TABLE 2.—NITROGEN BALANCE FIGURES DURING PARENTERAL ADMINISTRATION OF AMINO ACIDS TO 2 PATIENTS AFTER OPERATION.

Case number.	Period.	Diet.		Blood transfusion.		Amino acids.		Total nitrogen intake.	Urine output.		
		Protein, gm	Nitrogen, gm.	Protein, gm.	Nitrogen, gm.	Amount, cc.	Nitrogen.		Total nitrogen.	Amino acid nitrogen.	Creatine.
8	Exp. 8 days	..	..	..	..	500	5.0	5.0	8.58	2.8	679 1st 4 days 143 2d 4 days
	Control 4 days	..	..	..	..	..	..	..	3.96	2.25	71
	Control 6 days	20.3	3.23	..	..	..	..	3.23	3.68	1.9	92
9	Control 2 days	2.21	0.35	..	..	..	..	0.35	1.56	1.2	214
	Exp. 6 days	2.94	0.47	22.62	3.62	500	5.0	9.09	1.38	1.1	94
	Exp. 6 days	1.36	0.20	22.62	3.62	750	7.5	11.32	3.92	1.5	139
	Control 4 days	19.47	3.09	..	..	..	..	3.09	3.63	1.8	132

Case Reports. CASE 1.—Male, aged 60, was studied for 5 weeks. Following three control periods of one week each, subcutaneous injections of 500 cc. of the amino acids mixture were given daily for 7 days. This was followed by another control period of 7 days. The average total urine nitrogen output of the experimental period was 3.7 gm. higher than the average during the control periods. The amino acid nitrogen output during the experimental period showed a surplus of 0.3 gm. compared to the control periods. This means that about 6% of the injected mixture spilled over into the urine and that 94% (4.7 gm. N) was utilized daily. The urine creatine and creatinine showed no significant difference between the control and the experimental periods and the patient's weight remained constant.

CASE 2.—Male, aged 65, was studied for 3 weeks. Subcutaneous injections of 600 cc. of the amino acids mixture were given daily for 7 days after a control period of 8 days, and this was then followed by a second control period of 7 days. The average amino acid nitrogen spilled during the experimental period was 1.25 gm. more than that spilled during the control periods. This amount represents 20.8% of the amino acids intake; the remaining 79.2% (4.75 gm. N) was utilized. The daily output of nitrogen in the urine was below the intake of 12.4 gm. The creatine output varied from an average of 56 mg. daily during the control periods to 105 mg. during the experimental period. This indicated the loss of some body protein during the period of injections.

CASE 3.—Male, aged 75, was studied for 5 weeks. There were two experimental periods of 7 days each during which daily subcutaneous injections of 500 cc. were given, and there were three control periods of 7 days each

alternating with the experimental periods. The average amino acid nitrogen spilled during the experimental periods was 0.87 gm. more than the average spilled during the control periods. Therefore, 82.6% (4.13 gm. N) of the amino acids mixture was utilized. The average creatine and creatinine output in the urine was essentially the same throughout the periods of study. This excludes the possibility of any loss of body protein. The patient's weight increased from 54.5 kg. at the start to 59.3 kg. at the end of the study.

CASE 4.—Male, aged 28, was studied for 5 weeks. There were two experimental periods of 7 days each alternating with 3 control weeks. The average amino acid nitrogen spilled during the experimental periods was 0.5 gm. (10%) more than the average during the control periods, indicating that 90% of the amino acid nitrogen injected was utilized. The average urine creatine and creatinine output was essentially the same throughout the periods of study. The patient's weight increased from 68.6 kg. to 73.0 kg. during the study.

CASE 5.—Male, aged 51, studied for 3 weeks. There was an experimental period of 7 days preceded by and followed by a control week. During the experimental period 600 cc. of the amino acids mixture was given subcutaneously. The average amino acid nitrogen spilled during the experimental period was 0.65 gm. more than during the control periods. This was about 10% of the amino acid injected, indicating that 90% of the mixture was utilized. The average urine creatine and creatinine output was the same during the control and the experimental periods. The patient's weight remained the same.

CASES 6 and 7.—Two normal men who were given 500 cc. of the amino acids mixture intravenously. They were studied for 3 weeks—one experimental week with a week before and one after as controls. Case 6 showed an average amino acid nitrogen surplus during the experimental period of 0.86 gm., or 17% of the total amino acids mixture intake. Therefore, 83% (4.14 gm. N) of the amino acid injected was utilized. The patient was in good nitrogen balance. The urine creatine and creatinine remained the same throughout the studies. The patient's weight remained the same.

Case 7 showed an average amino acid surplus of 1.09 gm. during the experimental period. This was 22% of the amino acids mixture injected, indicating that 78% (3.91 gm. N) was utilized. The patient was in nitrogen balance and the urine creatine remained the same throughout the studies.

CASES 8 and 9.—These were 2 postoperative cases who were given intravenous infusions of the amino acids mixture. Case 8 was a woman with paralytic ileus of 6 days' duration before the injections were begun. No food was given by mouth; the patient received only 5% glucose in saline. Amino acids mixture (500 cc.) was given daily for 8 days. A control period of 4 days without food followed, and then a second control period of 6 days during which she took a diet containing 20.3 gm. protein. The amino acid nitrogen output during the experimental period was 0.75 gm. (or 15% of the total injected) more than during the control period with food, and 0.55 gm. more than during the period without food. Therefore, 85% of the amino acids mixture was utilized comparing the experimental period with the food period, and 89% comparing the experimental period with the control period without food. The creatine output in the urine, which was very high at the beginning of the study (600 to 1000), showed an abrupt decrease after 4 days of the injections. The patient was very toxic at the beginning of the study.

Case 9, the second postoperative case, was a female, aged 45, who had had a total hysterectomy. About 5 weeks later a cul-de-sac abscess was opened. Two days after this the amino acids mixture injections were begun. She was given 500 cc., diluted with distilled water to 1000 cc., in-

travenously every day for 6 days. She continued to have a high temperature, was vomiting and unable to retain food. She was then given intravenously 750 cc. of amino acid, diluted with an equal amount of distilled water, daily for 6 days.

Comparing the amino acid nitrogen output of the experimental with that of the control periods, there was no increased spill of amino acids. This means that all of the amino acids were utilized. The creatine in the urine decreased rapidly after the first control period of 2 days, in spite of a febrile period during the experimental periods, indicating that little body protein needed to be utilized when amino acids were administered.

Conclusions. 1. A mixture of amino acids containing all the essential amino acids has been prepared which can be administered to normal and postoperative patients subcutaneously or intravenously without untoward reactions.

2. Both subcutaneous and intravenous methods of injection were found to be efficient.

3. The parenteral administration of amino acids mixture could be substituted for protein in the diet to maintain the patient in nitrogen balance. In postoperative cases where food intake is not possible, the amino acid mixture was almost completely utilized and aided toward maintaining a nitrogen equilibrium.

The possibility of using these mixtures in other kinds of clinical conditions where peroral nutrition is not possible is to be investigated.

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VITAMIN C NUTRITION IN PELLAGRA.

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Pathologic states due to vitamin deficiency may be the result of the absence from the diet of one or of several essential substances.

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A multiple hypovitaminosis is probably more common than are beriberi, pellagra, or scurvy. Even these definite syndromes may be complicated by the deficiency of some other vitamin than the one primarily responsible for the disease. The diet of patients with pellagra in our clinic appeared to be extremely low in ascorbic acid. A similar observation has been reported by Spies.[5] It seemed likely that a deficiency of vitamin C would be of frequent occurrence in pellagra. Eighteen patients with this syndrome were accordingly studied in regard to the state of vitamin C nutrition. The amount of ascorbic acid in the blood plasma in the postabsorptive state was determined in each individual. An intravenous tolerance test was performed in 10 instances and an oral tolerance test in 4 others.

Method. The intravenous tolerance test was carried out as follows: The level of ascorbic acid in the plasma was determined before the intravenous administration of 1 gm. of crystalline ascorbic acid,* and at 20 minutes, 2, 4, and 6 hours following this administration. The urinary excretion in the subsequent 5 hours was likewise measured as suggested by Wright.[7] The oral tolerance test was performed as described by Goldsmith and Ellinger:[2] 600 mg. of ascorbic acid were given by mouth, the amount excreted in the urine in the subsequent 6 hours measured, and the level in the blood determined at the end of 1 and of 3 hours. The ascorbic acid in the blood was determined by the method of Farmer and Abt,[1] that in the urine by titration with 2-6-dichlorphenol-indophenol after acidification with glacial acetic acid.[2]

Of the 18 patients in this series, 12 had severe pellagra with characteristic glossitis and dermatitis, lesions of the alimentary and genito-urinary tracts, and manifestations of central nervous system disturbance. Three patients had glossitis and dermatitis of mild degree; 2 showed only the characteristic dermatitis; and 1 only the mucous membrane lesions, including glossitis, proctitis, and vaginitis, all of which were severe. In one instance, pellagra was complicated by a chronic gastric ulcer, in another by Parkinson's syndrome. In no case was a history of chronic alcoholism obtained. The diet, in most instances, had consisted largely of rice, red beans, cereals, milk, occasional salt meat and turnip greens; fresh fruit, vegetables, and fresh meat were rarely included.

The amount of ascorbic acid in the blood during fasting varied from 0.05 to 1.15 mg. per 100 cc. (Table 1). The average of the determinations in 17 cases (1 specimen lost) was 0.29 mg. Chart 1 shows the distribution of the findings. Less than 0.4 mg. of ascorbic acid per 100 cc. of blood, the minimal level which may be considered normal, was found in 13 instances. Eleven of these values occurred in persons with severe pellagra, 1 in the patient with lesions limited to the mucous membranes, and 1 in a relatively mild pellagrin. Two individuals showed a normal amount of vitamin C in the blood; that is, 0.74 and 1.15 mg. per 100 cc. Both had mild pellagra of short duration.

* Ascorbic acid supplied by Merck & Co.

The results obtained with the intravenous tolerance test corroborated in most instances the findings obtained on examination of the blood during fasting. A tolerance test, however, gives a much

TABLE 1.—INTRAVENOUS AND ORAL TOLERANCE TESTS OF VITAMIN C NUTRITION IN PATIENTS WITH PELLAGRA.

Case No.	Ascorbic acid in blood (fasting), mg. per 100 cc.	Vitamin C content after intravenous administration of 1000 mg. of ascorbic acid. Blood, mg. per 100 cc. 20 min.	2 hrs.	4 hrs.	6 hrs.	Urine output, mg. in 5 hrs.
1	0.09	6.10	2.52	1.12	0.50	221.0
2	0.12	7.81	1.66	0.84	0.44	
3	0.32	9.45	3.25	1.46	1.01	584.0
4	0.17	6.87	2.97	2.02	0.65	410.0
5*	0.09	6.50	3.91	1.54	1.32	122.6
6	0.22	8.10	1.54	0.97	0.92	261.0
7	0.27	...	1.98	1.60	..	228.0
8	..	11.67	3.66	1.61	1.94	528.0
9	0.17	6.32	1.70	0.65	0.61	100.0
10	0.41	12.25	4.66	1.89	1.45	322.0

Case No.	Ascorbic acid in blood (fasting), mg. per 100 cc.	Vitamin C content after oral administration of 600 mg. of ascorbic acid. Blood, mg. per 100 cc. 1 hr.	2 hrs.	3 hrs.	6 hrs.	Urine output, mg. in 6 hrs.
11	0.74	1.99	..	2.72	2.00	27.0
12	0.09	0.27	..	0.40	0.50	2.60
13	1.15	..	2.11			
14	0.05	0.06	..	0.05	0.12	0.62
15	0.35					
16	0.04					
17	0.42					
18	0.19					

* Prior to test 300 mg. of ascorbic acid was given daily by mouth for 10 days.

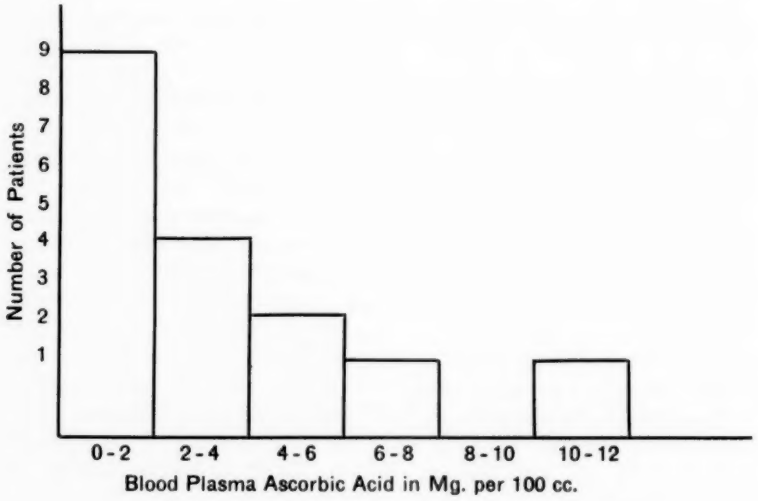

CHART 1.—Ascorbic acid in blood plasma in pellagra.

more satisfactory index of the degree of saturation of the tissues with vitamin C than does a single examination of the blood.

The response to the intravenous dose of vitamin C may be determined by measuring the excretion in the urine during a 5-hour period or by examination of the blood at intervals for 4 hours. It is our opinion that evaluation is more accurate if both the urine and blood are studied. In the presence of kidney damage, excretion of ascorbic acid may be delayed, with consequent retention in the blood.[6] A low renal threshold for ascorbic acid could lead to excessive output of this substance.[4] In either of these circumstances, determination of the ascorbic acid in the urine alone would lead to erroneous conclusions. Wright[7] and Portnoy and Wilkinson[3] have found that a normal response to the administration of 1 gm. of ascorbic acid by vein is the excretion of 400 mg. or more in the urine in 5 hours. Our studies are in agreement with these observations. Portnoy and Wilkinson[3] reported that the average levels of ascorbic acid in the blood in 10 normal persons after getting 1 gm. intravenously were 10.12 mg. per 100 cc. at 18 minutes, 2.76 mg. per 100 cc. at 2 hours, and 1.55 mg. per 100 cc. at 4 hours. The findings in 24 tests performed by us, 10 of which are reported here, have led us to conclude that when vitamin C nutrition is normal, the ascorbic acid in the blood rises to more than 7.0 mg. per 100 cc. in 20 minutes, remains above 2.5 mg. per 100 cc. at the end of 2 hours, and above 1.6 mg. per 100 cc. at the end of 4 hours.

The results of the intravenous tolerance test in 10 patients with pellagra (Table 1) indicated, as judged by excretion of ascorbic acid in the urine, a normal state of nutrition in 3 instances (Cases 3, 4, 8), an abnormal one in 6. The amount of ascorbic acid in the blood during fasting was subnormal in 2 tests in which the urinary excretion was normal (Cases 3, 4). This could be explained by recent inadequacy of the diet or failure of absorption from the intestinal tract of short duration, so that the stores in the body were not yet depleted. It is recognized that the level of vitamin C in the blood falls rapidly when the daily supply is diminished. The results of the intravenous tolerance tests, as judged by changes in the amount of ascorbic acid in the blood, indicated a normal state of vitamin C nutrition in 4 instances, a deficiency in 6. In Case 10, the urine value was at variance with what would be expected in view of the blood values. It is our opinion that subnormal ascorbic acid excretion in the urine in this case was due to inadequate fluid intake, since only 92 cc. of urine was excreted in 5 hours. Mild renal damage was not completely excluded as a causative factor. Nevertheless, the studies of the ascorbic acid in the blood were accepted as indicating a normal degree of saturation of the tissues with vitamin C in this individual.

An oral tolerance test was carried out in 4 patients with pellagra. When vitamin C nutrition is normal, the ascorbic acid in the blood

increases to 2.0 mg. per 100 cc., or more, 1 to 3 hours after giving 600 mg. by mouth, and the excretion in the urine is greater than 50 mg. in 6 hours. Two of the 4 patients (Cases 11, 13) were normal in regard to vitamin C nutrition, as judged by these criteria. In Case 11 there was evidence, from both clinical and laboratory standpoints, of diminished renal function, explaining the small quantity of ascorbic acid excreted in the urine when the level in the blood was well above 2.0 mg. per 100 cc.

In summary, a normal degree of tissue saturation with vitamin C was found in only 6 of 14 patients with pellagra, in whom intravenous or oral tolerance tests were performed.

The importance of recognizing multiple vitamin deficiency and of instituting adequate treatment is illustrated by 3 persons who were studied 3 to 6 months after recovery from pellagra. One returned to the hospital complaining of weakness, anorexia, and loss of weight. The second had vague gastro-intestinal complaints, and the third had purpuric areas in the skin. All 3 of these patients were found to have a vitamin C deficiency. The level of ascorbic acid in the blood during fasting was less than 0.2 mg. per 100 cc. in each case. An intravenous tolerance test in 2 of the patients and an oral tolerance test in the third indicated a marked deficiency of vitamin C (Table 2). There was rapid clinical improvement in each instance when a diet high in vitamins was instituted, supplemented by ascorbic acid, 200 to 300 mg. daily.

TABLE 2.—INTRAVENOUS AND ORAL TOLERANCE TESTS OF VITAMIN C NUTRITION IN PATIENTS WHO RECOVERED FROM PELLAGRA AND SUBSEQUENTLY DEVELOPED VITAMIN C DEFICIENCY.

Case No.	Ascorbic acid in blood (fasting), mg. per 100 cc.	Vitamin C content after intravenous administration of 1000 mg. of ascorbic acid. Blood, mg. per 100 cc. 20 min.	2 hrs.	4 hrs.	6 hrs.	Urine output, mg. in 5 hrs.
19	0.11	8.45	2.00	1.35	0.40	170.21
20	0.17	2.52	2.26	1.65	1.64	272.00

Case No.	Ascorbic acid in blood (fasting), mg. per 100 cc.	Vitamin C content after oral administration of 600 mg. of ascorbic acid. Blood, mg. per 100 cc. 2 hrs.	Urine output, mg. in 6 hrs.
21	0.04	0.86	0.85

Summary. The state of vitamin C nutrition was studied in 18 patients with pellagra. An ascorbic acid deficiency was found in 8 of 14 patients on whom intravenous or oral tolerance tests were performed. Four additional pellagrins showed less than the normal amount of ascorbic acid in the blood during fasting. Three persons who had recovered from pellagra and who later presented symptoms suggestive of a deficiency state were found to have a depletion of the vitamin C stores of the body.

Conclusion. Pellagra is often accompanied by a vitamin C deficiency. The therapeutic regimen in this condition should include a diet adequate in all the essential food factors, supplemented by ascorbic acid when a deficiency of this substance is demonstrated.

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COMPARATIVE STUDY OF THE BOERNER-LUKENS COMPLEMENT FIXATION TEST.

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Even the casual reader of current medical literature is forced to note the large number of "new" serologic procedures, as well as modifications of accepted procedures which are more or less constantly appearing. While many of these are described as "new," those familiar with serology will recognize that, in the last analysis, all the various complement fixation and flocculation procedures at present in use, and those proposed for use, as well as their modifications must necessarily, if they are to prove safe as well as efficient, rest upon the broad basic principles established by the laborious studies of a host of investigators.

Practically every "new" procedure advanced makes its appearance on one of two grounds, rapidity or simplicity in its performance. While these, in themselves, are desirable, they cannot—and should not—be regarded as the ultimate in desirability nor the sole nor even the outstanding criteria governing unquestioning acceptance. This should be emphasized, for it may well be feared that, to some extent, the fact that a serologic procedure is proclaimed as exceptionally rapid or essentially simple in its technical requirements has led—or may well lead—to its adoption and performance by those not always basically well prepared to assume the responsibility for the serologic diagnosis of syphilis. This is particularly true because of the present general interest in this disease and should be remarked upon and guarded against in order to lessen, as far as may be, the otherwise inevitable and—for the patient—disastrous aftermath.

Neither the competent clinician, the syphilographer nor the serologist will deny that the positive serologic reaction—when properly controlled and in competent hands—constitutes the most delicate and constant *single* symptom of syphilis. On the other hand, neither will they deny that when incompetently carried out, improperly interpreted or clinically misapplied the single serologic report constitutes the greatest single menace to both the recognition and the management of this disease.

Rapidity and relative technical simplicity are desirable assets, especially where the number of tests to be made is large. But it may again be said that they should not constitute *per se* the sole, nor even the outstanding criteria for the acceptance of a particular procedure.

The all-important criteria are only to be determined by the comparison of a new technique under proper control with methods which the test of time and trial have shown to be of acceptable delicacy and specificity.

Among the more recent modifications of the complement fixation test for syphilis is that proposed by Boerner and Lukens,[1] the advantages of which it is claimed include reduction in the time and labor required to set up and complete the test, and ready detection and avoidance of technical errors as well as sensitivity equal to procedures generally recognized and accepted, if not superior to some in general use.

Being among those who are firmly convinced that the safe and satisfactory serologic study of syphilis should include the application of both a complement fixation test and a flocculation test to each serum under study; recognizing that the acceptable complement fixation procedures in common use involve the expenditure of both time and labor; and recognizing also, that under some circumstances—as in boards of health, for example—time and labor are often factors of decisive importance, we were interested in the Boerner-Lukens procedure and determined to subject it to comparative trial.

It is unnecessary to add to the length of this communication by including a description of the minutia of the Boerner-Lukens method which is fully described in their original paper.

It is the purpose of this communication to report the results of its comparative trial in 1000 routine, unselected sera subjected at the same time to the Kolmer quantitative complement fixation test and the Kline flocculation test, both of which constitute the routine procedure of these laboratories.

While Boerner and Lukens describe both a qualitative and quantitative method, we chose to compare the quantitative method, for several reasons.

In the first place, our routine complement fixation is a quantitative test; in the second place, we have long been convinced by

extensive experience that the quantitative complement fixation report is of definite clinical value and utility. In fact, we are convinced that this point need no longer be labored.

The sera utilized in this study were from various sources: (*a*) sera routinely tested from cases from the wards and clinics of the Atlantic City Hospital; (*b*) sera from the Municipal Hospital Venereal Disease Clinic, many being known cases of syphilis under treatment; (*c*) sera from the Atlantic County Hospital for Mental Diseases.

In all 1000 sera were tested by all 3 procedures (Kolmer quantitative and Boerner-Lukens complement fixation tests and the Kline diagnostic flocculation test).

Before discussing the actual results of the comparison it may be said that, as compared to the Kolmer quantitative procedure, the Boerner-Lukens method is economical in the time required to set up an equal number of tests. It is also claimed for the latter method that, because, through consolidation of various constituents of the test, the volumes measured are relatively large, as a result, technical errors are lessened and, because of the equal volume in all tubes, if present are readily detected.

The latter is also true of the Kolmer test and, in the last analysis, as long as technical manipulations in complement fixation tests should always be governed by concentrated cerebration—as we believe to be the case—these factors, while valuable, are, after all, of relatively minor importance.

The important factors are the relative delicacy and sensitivity of the various procedures.

While comparative studies are commonly presented in tables and graphs—and, perhaps, best so presented—we believe that the salient features of the present study are evident from the brief summations below at the saving of considerable space:

Kolmer positive reactions in 1000 sera: 16.7%
Kline positive reactions in 1000 sera: 15.4%
Boerner-Lukens positive reactions in 1000 sera: 17.3%
Percentage agreement between Kolmer and Kline: 91.3%
Percentage agreement between Kolmer and Boerner-Lukens: 84.9%
Percentage agreement between Boerner-Lukens and Kline: 87.1%

The quantitative comparison between the complement fixation tests, both set up with 5 test doses of serum, was as follows:

In 83% of the positive reactions both tests showed fixation in the same number of tubes; in 17% the number of tubes showing fixation varied as shown below:

	Kolmer positive (167).	Boerner-Lukens positive (173).
Fixation in 5 tubes	36	78
Fixation in 4 tubes	30	33
Fixation in 3 tubes	53	37
Fixation in 2 tubes	32	17
Fixation in 1 tube	16	8

From this it would appear that while the number of very strongly positive (5-tube fixation) was greater by the Boerner-Lukens test, there were more weakly positive reactions more definitely shown by the Kolmer test while the difference between the total number of positive reactions detected was small (6).

Of the sera negative to the Kolmer test 0.25% were positive to the Boerner-Lukens test and 0.04% were positive to the Kline test.

Of the sera negative to the Boerner-Lukens test 0.17% were positive to the Kolmer test and 0.08% were positive to the Kline test.

Of the sera positive to both complement fixation tests 0.09% were negative to the Kline flocculation test.

Considering the positive reactions as a whole, the Kolmer test detected 1.3% more positive reactions than the Kline test, while the Boerner-Lukens test detected 1.9% more positive reactions than the Kline test and 0.6% more than the Kolmer test.

We do not believe that from differences such as these any far reaching or conclusive inferences may be drawn concerning the relative sensitivity of the methods in question.

For, as the experience of serologists in general has decisively demonstrated, it is practically impossible to obtain complete and entire agreement between any two serologic procedures simultaneously applied to any extensive series.

This, it is rather generally believed, results more from inherent variations in the affinity of the serum lipids for the lipids in various antigen suspensions than from any inherent or marked variation in the sensitivity of the methods in question which is only to be inferred when such differences in the reactions obtained are both numerous and marked.

Our purpose in this study was not to determine differences in sensitivity but rather to ascertain if the Boerner-Lukens method compared with two methods shown by our own experience, as well as by the general experience of many others, to possess an acceptable degree of delicacy and sensitivity.

One of the difficulties apt to be encountered when specimens are received from various sources, under various conditions and in varying stages of preservation, is the occurrence of anticomplementary reactions. In our experience with the Kolmer test the incidence of such reactions has consistently been less than 1% and it is of some interest that in this series the number of anticomplementary sera (11) was the same for both complement fixation procedures.

Summary. From this study we believe that, in addition to being economical in reagents, time and labor, the Boerner-Lukens modification of the complement-fixation test is shown to possess a degree of sensitivity and delicacy warranting its inclusion among the procedures acceptable for general use in the serologic study of syphilis.

We believe, further, that Department of Health Laboratories

which hitherto may have omitted complement fixation tests for syphilis for the principal reason of the time and labor expended will find the Boerner-Lukens technique, especially in its qualitative form, well suited to their needs.

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ACUTE LYMPHOCYTIC CHORIOMENINGITIS.

REPORT OF THREE CASES WITH HISTOPATHOLOGIC FINDINGS.

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IN 1931 Gibbens[4] directed the attention of the medical world to a group of unusual and little understood infections of the central nervous system, especially one described under various names, but which in the absence of definite knowledge he preferred to call acute aseptic meningitis. Although described by Wallgren in 1925 as "a new infectious disease of the central nervous system," Gibbens states that as early as 1910 14 cases were published in Paris under various titles such as "syndrome meningé avec lymphocytie rachidienne d'origine indéterminée" (Laubry and Foy) and the like.

According to Collis,[2] since the important communication of Wallgren a large number of cases presenting a similar syndrome have been described in various countries of Europe and America. Hughes[5] reported 2 cases in the Malayas which he claimed to be representative of half dozen other cases, Asiatic as well as European. In his opinion the incidence in the Malayas is comparable to that of European countries; all races being equally affected on the peninsula.

The syndrome is characterized by its acute onset, with definite meningeal signs, including changes in the cerebrospinal fluid, such as increased fluid and pleocytosis (predominantly lymphocytes), coupled with little or no change in the fluid sugar and protein; absence of known pyogenic infections, especially otitis media, sinusitis, and so on; and a usually short course and favorable termination.

The relatively benign course as well as the failure to demonstrate the etiologic agent, besides causing considerable confusion in the nomenclature, has hitherto caused these cases to be looked upon rather lightly. In 1935, Collis declared that these cases should no longer be regarded as medical curiosities, and advanced the proba-

bility that the etiologic agent may have been a virus until then undiscovered. This question of a virus etiology has received a great deal of consideration; but is now generally considered satisfactorily answered in favor of the virus isolated by Armstrong and Lillie in 1934,[1] and later by other experimenters in this country as well as in Europe. It has been cultivated on the chorio-allantoic membrane of the chick embryo. Its recovery from all tissues and organs of experimental animals points to it as a systemic rather than entirely a local infecting agent.

The diagnosis is made chiefly on the clinical findings, and clinched by serologic evidences of the presence of the Armstrong virus in the spinal fluid. Tuberculous meningitis is ruled out chiefly by the absence of the tubercle bacillus, and the relatively undisturbed sugar and protein content of the cerebrospinal fluid; also the relatively benign course and favorable termination. Non-paralytic poliomyelitis, according to Lucchesi,[6] is clinically indistinguishable from an uncomplicated syndrome of lymphocytic choriomeningitis. The same may be said of mild cases of encephalitis, except, perhaps, where epidemics of these more serious conditions exist. In case of doubt, however, the virus (where possible) may be identified by its immunologic characteristics.

The literature dealing with complications and sequelæ has been reviewed by Skogland and Barker.[9] Mention has been made of residual involvement of the central nervous system, including periodic headaches, dizziness, strabismus, fatigue, forgetfulness, personality changes, Parkinsonism, chronic arachnoiditis with spastic paraplegia, and chronic meningoencephalitis.

With regard to histologic changes, most of our information has been obtained from experiments on mice and monkeys. Recently a few human cases have been reported with necropsy findings. In 1934 Gallet[3] published his paper entitled "Méningite à liquide clair et à lymphocytes, comateuse, d'emblée mortelle, chez une ancienne méningite lymphocytaire." Unfortunately, no histologic studies were made and the diagnosis was never confirmed. In 1934, Riggs[8] presented a case to the Philadelphia Neurological Society in which meningoencephalitis was present. Viets and Warren[10] were apparently the first to report a fatal case with detailed studies of histologic changes. According to their findings both meningitis and encephalitis were present. The characteristic changes included lymphocytic infiltrations, mostly perivascular; also petechial hemorrhages, glial nodules and large swollen ganglion cells filled with inclusion bodies and pigmented granules. The reaction was most marked in the regions of the midbrain and pons. In 1939, Machella, Weinberger and Lippincott[7] reported another fatal case with autopsy findings, the essential histologic changes including marked thickening of the meninges due to connective tissue proliferation with obliteration of the subarachnoid spaces. The meninges were infiltrated with

lymphocytes, red blood cells, and macrophages. The ependyma of the entire ventricular system was denuded and fragmented, the walls studded with ependymal granulations, and a narrow subependymal zone the site of intense inflammatory reaction. The choroid plexus was partly necrotic and heavily infiltrated with inflammatory exudate. In the floor of the fourth ventricle the process was sufficiently severe to cause softening involving especially the acoustic tubercle and right medial vestibular nucleus. The authors considered unusual the fact that while the meninges, ventricular walls, and choroid plexuses showed intense inflammatory reaction, the remainder of the brain substance was essentially normal.

It is obvious to those acquainted with the present literature that owing to the scarcity of necropsy material the extent and character of the histologic changes in acute lymphocytic choriomeningitis are in need of further clarification. Cases coming to necropsy with the clinical diagnosis of lymphocytic choriomeningitis therefore assume corresponding importance. Accordingly, 3 such cases are here reported.

Case Reports. CASE 1.—C. C., a 72-year-old white male, was brought to the Colorado General Hospital on September 18, 1939 by his niece, with the complaint that 3 days before he developed a sore, painful back. He complained a great deal, but continued to work until the next day when he became irrational, following an attack of severe headache. The day before admission he developed peculiar muscular movements, such as clutching at the bed clothes and bicycle riding, and finally had to be restrained. His past history showed that on August 30, 1936, he was previously hospitalized because his prostate was enlarged and painful, but he was discharged on September 18, 1936, improved.

Upon admission the temperature was 102.4° F., pulse 100, and respiration 25. The blood pressure was 152/85.

When examined, he was lying quietly in bed, apparently awake, but unresponsive. He gave the impression of being quite ill. The skin was hot and dry, and gave evidence of much weight loss. His constricted pupils reacted slowly to light. The retinal arteries showed marked crossing signs and silver-wire effects. Ears, nose and mouth showed nothing of particular interest. The neck was held rigid and could not be flexed; lateral movements not restricted. The chest was apparently in fairly good condition. On previous hospitalization, August 30, 1936, the electrocardiogram was normal. Abdomen and external genitalia were essentially negative, but the prostate was approximately three times the normal size. It was smooth, firm and unfixed. Reflexes were hyperactive. There were present a right Babinski, bilateral Kernig and Brudzinski signs.

The clinical impressions were: lymphocytic choriomeningitis, generalized arteriosclerosis, and prostatic hypertrophy. Laboratory findings: The urine showed specific gravity of 1.016, was otherwise negative. Blood: hemoglobin, 15.5; red blood cells, 5,850,000; white blood cells, 14,000; hemogram normal. Sedimentation rate was 4% in ½ hour; 6% in 1 hour. The Wassermann reaction was negative. Blood sugar was 94 mg. per 100 cc.; non-protein nitrogen, 84 mg.; urea, 63; and creatinine, 2.5 mg. per 100 cc. Spinal fluid findings on September 20, 1939, were: pressure,

300 mm. of water; cells, 100 (lymphocytes and a few red cells); protein, 70 mg.; sugar, 53 mg.; Wassermann, negative; colloidal gold curve, 0001 10000.

The patient was given 3000 cc. of 10% glucose in Ringer's solution, covered by 20 units of old insulin. This was done repeatedly in efforts to restore fluids, supply nutrition and overcome the consequences of dehydration. However, the hospital course was predominantly downhill. Consciousness was never regained. His temperature fell to 98.4° F. on the third day, but soon climbed back to 103° F. It fell again on the fifth day, but rose steadily back to 104° F., when he expired. Diagnosis: acute lymphocytic choriomeningitis.

Autopsy findings: The brain weighed 1475 gm. after embalming. The leptomeninges were slightly thickened, but glistening; no exudate noted. Vessels on the base were greatly thickened, dilated, and showed many yellowish plaques and partly calcified areas. On section, the lateral ventricles were found to be widely dilated. Structures of the gray and white matter appeared to be without hyperemia, softenings or hemorrhages. In the lower right half of the midsection of the pons a couple of old cysts, probably due to softening, were seen, the only ones noted.

The histologic changes were quite impressive. The leptomeninges showed patchy lymphocytic infiltrations, sometimes perivenous, and generally of moderate degree. This was more pronounced along the brain-stem region, both in the arachnoid spaces and in the meshes of the pia. In all regions of the brain, however, some areas appeared to have been spared. Incidentally, the infiltration as a rule failed to overlap the adjacent gray matter. An unusual amount of melanotic pigment was found to be present in the basal meninges. A very moderate encephalitis was observed in extracortical regions, including the basal ganglia, substantia nigra, pons, olivary nucleus, and cerebellum. This was characterized by lymphocytic infiltrations (chiefly perivenous), noticeably absent from the cortex and white matter, and, incidentally, from the plexuses as well. Glia stars appeared mostly in the cerebellum, olivary nucleus, substantia nigra, and to a less degree in the cortex and white matter. Shrub-like proliferations also were observed in the cerebellum. The substantia nigra, while generally well preserved, exhibited foci of black pigment scattered about the nerve cells or ingested by glial phagocytes. In addition to the meningitis and encephalitis there was present some arterio- and arteriolosclerosis; but areas of softening were lacking except for those in the pons already mentioned. Diagnosis: lymphocytic choriomeningitis with moderate encephalitis. In other organs the main findings were: confluent bronchopneumonia in lower half of both lungs, hypertrophy of the heart, moderate nephrosclerosis, hypertrophy of the prostate, and generalized arteriosclerosis.

CASE 2.—C. J., a 67-year-old white male, was admitted to the Colorado General Hospital on August 21, 1938. Two weeks before, he had complained of numbness of both hands, occasionally severe headaches, and fever. Since that time there have been generalized aches and pains about the body with stiffness ascending to the neck. He was comatose when admitted, and there was rectal incontinence which had begun two days before. Temperature was 103.4° F.; pulse 96; and respiration rapid and labored. Past history was of little interest. Six years ago there was an attack of "nervous indigestion," followed shortly by pneumonia. Four years previous to this, a nervous breakdown was said to have resulted from financial stress. Examination revealed alopecia, a hot dry skin, slight neck rigidity, and limited chest expansion. The heart rate was rapid, but the rhythm regular. An apical systolic murmur and an accentuated pulmonic second sound were present. The rectal sphincter was relaxed; the prostate was apparently enlarged to palpation. There was moderate rigidity of all extremities. Peripheral vessels were hard and beaded; blood

pressure, 184/148. Deep reflexes were apparently increased; superficial reflexes diminished.

Laboratory findings were as follows: Urine, specific gravity 1.016, albumin 2, hyaline casts 4, coarse granular casts 2. Blood: hemoglobin, 15.2; red blood cells, 4,800,000; white blood cells, 10,000 (80% neutrophils); sugar, 134 mg.; non-protein nitrogen, 60 mg., and creatinine, 2 mg. per 100 cc. Spinal fluid: pressure normal, cells 40 (mostly lymphocytes), sugar 66 mg. and protein 41 mg. Wassermann reaction negative; colloidal gold curve 0001221000; no bacteria.

The temperature curve was predominantly febrile, with daily spikes. On August 27, 1938, his condition was obviously critical; temperature was 103° F.; respiration of the Cheyne-Stokes type. Oxygen was administered, but he expired on August 29, 1938, at 12.45 noon.

Autopsy: The gross findings were mainly as follows: stretching of the dura and slight thickening of the leptomeninges with globular thickenings of the subarachnoid membrane by accumulations of cerebrospinal fluid. The brain itself weighed 1140 gm. The cortex was atrophic, and there were numerous foci of partly cystic softenings of different sizes in right thalamus, left internal capsule, and pons. There were remainders of small hemorrhages in the left dentate nucleus and a globular fresh hemorrhage in the left frontal lobe. The ventricles were slightly dilated. The basal vessels were sclerosed, partially thickened, and contained many yellowish plaques in the intima. The microscopic findings were essentially those of a meningitis with encephalitis, the latter characterized by marked lymphocytic infiltrations around small vessels located chiefly in the substantia nigra and pons; predominantly in the locus cæruleus. Ganglion cells everywhere showed slight evidences of fresh degeneration, such as swelling, vacuolation and loss of Nissl substance. The corpus callosum too was involved in the form of dilated vessels with petechial hemorrhages, and perivascular lymphocytic infiltrations. The glial reaction was essentially in the form of scattered nodular proliferations. The leptomeninges showed patchy lymphocytic infiltrations, in the subarachnoid spaces as well as in the meshes of the pia. The plexuses were thickened by fibrous proliferations and contained many corpora amylacea and scavenger cells filled with fat and pigment. All changes were predominant in the pons and mid-brain regions. The arteriosclerotic changes were apparently incidental and not directly connected with the inflammatory process. Diagnosis: subacute lymphocytic choriomeningitis with encephalitis, arteriosclerosis.

Case 3.—A. S., a 79-year-old white female, was brought to necropsy with the clinical diagnosis of acute lymphocytic choriomeningitis. She had not been previously hospitalized.

Necropsy findings: The brain was found to be grossly atrophic. The basal vessels were sclerosed; the leptomeninges dull and thick. The white matter was decidedly pale. In contrast, however, the pons was slightly hyperemic. The histologic changes in the meninges were chiefly in the form of patchy lymphocytic infiltrations and moderate fibrosis. The lymphocytes were located predominantly in the meshes of the pia. Although these changes were most marked in the regions of the brain stem, the cortical furrows were well represented. Changes in the brain parenchyma were observed chiefly in the substantia nigra and locus cæruleus, characterized by perivascular lymphocytic infiltrations, mostly of the smaller vessels, with additional destruction of some few ganglion cells and focal glial proliferation. In other brain regions encephalitic changes were scarcely noted, whereas moderate degrees of senile involution were prevalent. Other organs: the most important findings here were bronchiectasis, nephrosclerosis, and generalized arteriosclerosis.

Discussion. Despite the fact that no attempt was made to identify the virus of Armstrong in any of the cases here presented, the clinical course and histologic brain changes undoubtedly justified the clinical diagnosis of acute lymphocytic choriomeningitis. Furthermore, as far as can be ascertained, there were no epidemics of poliomyelitis or encephalitis concurrent at the time that these cases were seen, absence of which is considered presumptive evidence in favor of the more benign condition. It may be of some interest to note also that of the few cases coming to necropsy, only in one instance did the histologic studies show uncomplicated involvement of the membrane structures (Machella and coworkers); a finding which was admittedly unexpected by the authors themselves. The presence of complicating encephalitis in the present cases is in agreement with the findings of other authors, especially Viets and Warren, who consider mild encephalitis an important part of the histologic picture. At the present time it is not definitely known whether the encephalitis precedes or follows the meningeal reaction. The pathway of infection is also open to further investigation.

The question of a close relationship between such virus infections as epidemic encephalitis, poliomyelitis and acute lymphocytic choriomeningitis has been the subject of much discussion. The prevalence of similarity in clinical as well as pathologic findings has led to the belief that there should be more than mere coincidence attached to these findings.

Although lymphocytic choriomeningitis is considered a disease of early life, middle and old age are not exempt. Of the few cases reported with autopsy findings, one (Machella and coworkers) was 14 years old, and another (Viets and Warren) was 20 years old. In the present series, ages ranged from 67 to 79. No doubt in the aged the syndrome of lymphocytic choriomeningitis may follow a somewhat different course from that seen in the young. Whether or not this be the case, it is reasonable to suppose that lowered vitality as well as the presence of intercurrent infection in the aged are factors which should affect the course in a manner which is most unfavorable. This report therefore can hardly affect the question of benignity in connection with lymphocytic choriomeningitis. The findings, however, are in agreement with those of other authors (Viets and Warren): namely, meningitis, characterized by lymphocytic infiltrations chiefly in the basal leptomeninges, and mild encephalitis in the extracortical regions.

The character of the meningeal reaction deserves some mention in this discussion. The patchy distribution and areas of predilection were in conformity with no other known type of meningeal infection. On the other hand, the intracerebral infiltrations closely resembled in character and distribution changes due to epidemic encephalitis. The predominant perivenous nature of the infiltrations also agrees

with that seen in other virus infections, such as in postmorbillous encephalitis, the significance of which is not clearly understood.

In conclusion, it should be emphasized that the senile changes, such as cortical atrophy, and the arteriosclerotic changes were incidental and quite independent of the inflammatory changes due to the lymphocytic reaction.

Summary. Three cases clinically diagnosed as acute lymphocytic choriomeningitis are reported with necropsy findings. In all 3 cases the histologic brain changes were essentially of an acute or subacute meningeal reaction with concomitant encephalitis.

The meningitis was characterized by patchy infiltrations (lymphocytic) of the leptomeninges, most marked in the neighborhood of the midbrain and pons. The encephalitic process was in all cases of moderate intensity, and in distribution corresponded closely with predilection centers of epidemic encephalitis.

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CERTAIN FACTORS GOVERNING THE INCIDENCE OF CEREBRO-VASCULAR CRISES

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An interest in the relationship between cardiac and intra-cranial lesions was aroused during a survey of the incidence of cerebro-vascular crises in association with coronary artery occlusion.[1] Protocols of 1000 consecutive autopsies performed at this hospital were analyzed and the clinical records studied to determine any particular factors which might govern the occurrence of cerebro-vascular accidents.

In this series the brain was examined in 138 instances. Autopsy in 29 other cases showing definite clinical evidence of cerebral lesions did not include brain examination; these are included as "cerebro-vascular lesions, type undetermined," thereby establishing a more nearly true cross-section of the group. As the great majority of lesions are related to the vascular system (Table 1), this group was selected for consideration in its relation to heart disease, hypertension, arrhythmia and syphilis, as well as age, sex and race.

TABLE 1.—CHIEF FINDING AT AUTOPSY.

	No. of cases.		Per cent of the total examined.
Cerebral thrombosis	77		55.8
Cerebral hemorrhage	25		18.1
Thrombosis and hemorrhage	4		2.9
Embolism	1		0.7
	—	107	
Cerebral thrombosis and myelomalacia of cord	1		0.7
Subdural hemorrhage	3		2.1
Subarachnoid hemorrhage	1		0.7
Extradural hemorrhage	1		0.7
Multiple sclerosis and old hemorrhage	1		0.7
Meningo-vascular syphilis	1		0.7
Marked cerebral arteriosclerosis	4		2.9
Old cortical scars	2		1.4
Edema and congestion	3		2.1
Meningitis (2 miliary abscess)	5		3.6
Abscess	2		1.4
Tuberculoma	2		1.4
Glioma	2		1.4
Metastatic carcinoma	1		0.7
Fibroblastoma	1		0.7
Cerebral atrophy (idiot)	1		0.7
	—	31	
Cerebral lesion—type undetermined	29		
Total	167		

It should be pointed out that, in the following discussion, the term "hypertensive heart disease" is restricted to those cases with hyperpiesis (elevation of systolic and diastolic pressure above 140 and 90 respectively), cardiac enlargement, clinical signs of heart disease without coronary sclerosis of note or myofibrosis; this group was composed chiefly of relatively younger individuals suffering with "essential" or malignant hypertension, or renal disease. "Arteriosclerotic heart disease" includes individuals with or without hypertension in whom the chief pathologic finding is coronary sclerosis and fibrosis of the myocardium, excluding cases of cardiovascular syphilis with focal scarring of the heart muscle.

Heart Disease. In the 343 cases of heart disease (Table 2), 116 (33.8%) were complicated by a cerebral lesion, the majority having hemorrhage and thrombosis, whereas the incidence of cerebral lesions in the entire group of 1000 cases was 16.7%. The tabulation indicates that the percentage of cerebral complications was about the same in hypertensive, arteriosclerotic, coronary thrombotic and syphilitic heart disease. The incidence in rheumatic heart disease and acute bacterial endocarditis was less, while subacute bacterial endocarditis and congenital heart disease were represented by too few cases to be of significance. Thus far, then, one may conclude that heart disease in general is a major factor in the occurrence of cerebro-vascular lesions.

Hypertension per se. Hypertension was noted in 21.6% of the entire group, and 52% of cardiac cases and 74.2% of individuals

with cerebral accidents were hypertensive (Table 3). A comparison of Tables 2 and 3 indicates that the types of heart disease which are more apt to be complicated by a cerebral lesion are those usually associated with hypertension. As it appears that hypertension is observed twice as frequently in cardiac patients, and cerebro-vascular accidents occur more than twice as commonly in association with cardiac disease, it is reasonable to believe that the hypertension *per se*, rather than the cardio-vascular disease proper (apart from embolic phenomena), is responsible for the cerebral lesions.

TABLE 2.—INCIDENCE AND TYPES OF CEREBRAL LESIONS IN EACH TYPE OF HEART DISEASE.

Heart disease (total No. 343).		Associated cerebral lesions.									
Type.	Number.	No. with cerebral lesion.	Per cent with cerebral lesion.	Cerebral thrombosis.	Cerebral hemorrhage.	Hemorrhage and thrombosis.	Embolism.	Miliary abscess.	Abscess.	Cortical scar.	Type undetermined.
Rheumatic	32	7	21.8	3	..	..	1	1	..	..	2
Cardiovascular syphilis	52	16	30.7	9	3	..	..	1	..	..	3
Subacute bacterial endocarditis	3	2	66.6	..	..	..	1	..	..	..	1
Acute bacterial endocarditis	17	2	11.8	..	..	..	..	2			
Hypertensive	39	12	33.3	8	3	..	..	..	1		
Arteriosclerotic	155	62	40.0	31	7	2	..	..	..	2	20
Coronary thrombosis	44	15	34.0	11	..	1	..	..	..	..	3
Congenital	1	0	0.0								

An attempt to determine the frequency of acute cerebro-vascular accidents following the use of vasodilators in hypertensive individuals was unsuccessful, due to lack of uniformity in certain of the clinical records.

TABLE 3.—INCIDENCE OF HYPERTENSION IN CASES WITH CEREBRAL VASCULAR LESIONS.

Type of cases.	No.	No. with hypertension.	Hypertensives with cerebral complication.
Rheumatic heart disease	32	7— 21.8%	7—100.0%
Cardiovascular syphilis	52	17— 32.7%	16— 94.1%
Subacute bacterial endocarditis	3	0— 0.0%	0— 0.0%
Acute bacterial endocarditis	17	4— 23.4%	2— 50.0%
Hypertensive heart disease	39	39—100.0%	12— 30.7%
Arteriosclerotic heart disease	155	94— 60.7%	62— 66.0%
Coronary thrombosis	44	19— 43.1%	15— 78.9%
Congenital heart disease	1	0— 0.0%	0— 0.0%
	343	180— 52.0%	114— 63.3%
Miscellaneous	657	36— 5.4%	10— 27.7%
Entire group	1000	216— 21.6%	124 (74.2% of 167)

Arrhythmias. Following the suggestion of several authors that arrhythmias may interfere with the cerebral circulation and even be responsible for hemiplegia, this series was analyzed from this standpoint. Of the 1000 cases, 4.3% had auricular fibrillation, while 3.5% of those with cerebral lesions were fibrillating. No case with auricular fibrillation associated with thrombosis of the left auricle or appendage showed an anatomic lesion of the brain, although the possibility of embolism following dislodgment of such thrombotic material is not questioned. There is no doubt, too, that arrhythmias are capable of producing transient cerebral manifestations, due to circulatory alterations with edema of the brain, sometimes of sufficient degree to contribute to or even cause death.

TABLE 4.—RELATION BETWEEN AURICULAR FIBRILLATION AND CEREBRAL LESIONS.

	Without auricular fibrillation.		With auricular fibrillation.	
Type of case.	Number.	No. with cerebral lesion.	Number.	No. with cerebral lesion.
General group	957	163—17.0%	43	6—14.0%
Rheumatic heart disease	21	7—33.3%	9	2—20.0%
Cardiovascular syphilis	52	16—30.7%	0	0— 0.0%
Subacute bacterial endocarditis	3	2—66.6%	0	0— 0.0%
Acute bacterial endocarditis	16	2—12.4%	1	0— 0.0%
Hypertensive heart disease	36	13—36.1%	3	0— 0.0%
Arteriosclerotic heart disease	131	62—47.3%	24	3—12.4%
Coronary thrombosis	38	15—39.4%	6	1—16.0%
Congenital heart disease	1	0— 0.0%	0	0— 0.0%

TABLE 5.—RELATION BETWEEN SYPHILIS AND CEREBRAL LESIONS.

	The Wassermann and percentage with cerebral lesion.			
Type of case.	Wassermann positive.	Wassermann negative.	No Wassermann history negative.	No Wassermann history positive.
Entire 1000	79—13.9%	445— 21.5%	27—14.7%	449—14.6%
Rheumatic heart disease	1— 0.0%	12— 16.6%	1— 0.0%	16—17.5%
Cardiovascular syphilis	22—36.3%	5— 0.0%	4—25.0%	18—22.0%
Subacute bacterial endocarditis	0— 0.0%	1—100.0%	0— 0.0%	1— 0.0%
Acute bacterial endocarditis	2— 0.0%	4— 25.0%	0— 0.0%	11— 9.0%
Hypertensive heart disease	2— 0.0%	27— 33.3%	0— 0.0%	10—40.0%
Arteriosclerotic heart disease	6—16.6%	81— 32.0%	0— 0.0%	50—30.0%
Coronary thrombosis	3— 0.0%	24— 29.0%	0— 0.0%	19—26.3%
Congenital heart disease	0— 0.0%	0— 0.0%	0— 0.0%	1— 0.0%
Cases with cerebral lesions	11	96	4	66

Syphilis. Serologic examinations for syphilis in 524 of the cases gave 79 positive and 445 negative results; 27 untested cases volunteered a positive history of syphilis. The incidence of cerebral lesions, in the presumably syphilitic individuals, was lower than in the remaining subjects, although fairly high (30.7%) in those with actual cardiovascular syphilis. It is difficult, however, to evaluate the presence of this disease in the group because of unreliable histories, certain numbers of falsely negative tests, and a scattering of

individuals Wassermann-negative after treatment. In a general way, though, it appears that syphilis is not a very important factor in the etiology of cerebro-vascular accidents.

Age. That age is an important factor is shown by the gradual, progressive increase in the incidence of cerebro-vascular lesions with advancing years (Table 6), the apparent fall beyond 80 being due to the relatively few cases studied. This is naturally the case with progressive sclerosis in the cerebro-vascular tree, often complicated by an hypertensive state. Cerebral lesions occur with greater frequency in the lower age groups with rheumatic heart disease; other types of heart disease are more apt to be thus complicated between the ages of 40 and 59 years.

TABLE 6.—INCIDENCE OF CEREBRAL LESIONS AT DIFFERENT AGES.

Type of case.	Age group and percentage with cerebral lesion.				
	Below 20 years.	20 to 39 years.	40 to 59 years.	60 to 79 years.	80 and above.
Entire 1000	88— 5.6%	200— 7.5%	406—12.5%	256—19.9%	50—16%
Rheumatic heart disease	6— 33.3%	11—18.0%	9—11.0%	6—33.0%	
Cardiovascular syphilis	....	6— 0.0%	32—34.0%	14—35.0%	
Subacute bacterial endocarditis	1—100.0%	2—50.0%			
Acute bacterial endocarditis	....	10—10.0%	5—20.0%	2— 0.0%	
Hypertensive heart disease	....	7—42.8%	28—32.0%	4—25.0%	
Arteriosclerotic heart disease	....	....	49—47.0%	99—33.0%	7—85%
Coronary thrombosis		5—60.0%	23—30.0%	15—33.3%	
Congenital heart disease	1— 0.0%				
Cases with cerebral lesions	5	17	68	67	10

TABLE 7.—COMPARISON OF THE INCIDENCE OF CEREBRAL LESIONS IN THE TWO SEXES AND IN THE WHITE AND COLORED RACES.

Type of case.	Sex and color and percentage with cerebral lesion.			
	Female.	Male.	White.	Colored.
Entire 1000	433—27.2%	567— 8.6%	626—19.1%	374— 8.8%
Rheumatic heart disease	20—30.7%	12— 0.0%	23—21.7%	9—22.2%
Cardiovascular syphilis	11—13.4%	41—29.2%	21—26.2%	31—29.9%
Subacute bacterial endocarditis	0— 0.0%	3—66.6%	3—66.6%	0— 0.0%
Acute bacterial endocarditis	10—20.0%	7— 0.0%	12— 8.9%	5—20.0%
Hypertensive heart disease	24—45.8%	15— 6.6%	21—42.8%	18—22.0%
Arteriosclerotic heart disease	77—45.4%	78—26.9%	117—32.4%	38—54.5%
Coronary thrombosis	18—22.2%	26—42.3%	26—42.3%	18—22.2%
Congenital heart disease	1— ...	0— ...	1— 0.0%	0— ...
Cases with cerebral lesions	118	49	120	47

Sex and Race. There were 567 males and 433 females in the series. Cerebro-vascular lesions occurred in 8.6% of males as opposed

to 27.2% of females, the 1 : 3 ratio in a thousand cases being regarded as significant. Whites outnumbered negroes 626 to 374, with cerebral accidents occurring in 19.1 and 12.6% respectively. It is interesting to note that the preponderance of cerebral lesions associated with cardiovascular syphilis appeared in males and that all females with cardiovascular syphilis were colored. Coronary thrombosis accompanied by cerebral lesions was more frequent in males, particularly whites between 40 and 59 years.

Summary. The clinical and autopsy records of 1000 consecutive, unselected cases were studied to determine any correlation between cerebro-vascular lesions and heart disease, hypertension, arrhythmia, syphilis, age, sex and race, the following conclusions being drawn:

1. Hypertension is probably the most important single etiologic factor in cerebro-vascular crises.
2. Heart disease in general is a major etiologic agent.
3. There is a gradual, progressive increase in incidence with advancing age, excepting the younger group associated with rheumatic heart disease.
4. Cerebro-vascular lesions occur 3 times as frequently in females as in males and nearly twice as often in whites as in negroes.
5. Syphilis *per se* has little influence; the percentage found with cardiovascular syphilis approaches that of heart disease in general.

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THE EFFECT OF PARTIAL HEPATECTOMY ON THE ACTION OF CERTAIN BARBITURATES AND A PHENYLUREA DERIVATIVE.

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THE MAYO FOUNDATION, ROCHESTER, MINN.

Chloroform and carbon tetrachloride are liver poisons commonly used to produce experimental hepatic damage. These substances have found considerable use in studying the protective function of the liver against certain noxious agents.

Pratt and his coworkers[13] noted that experimental liver injury prolonged the action of certain of the hypnotic drugs. They found a decreased tolerance to pentobarbital sodium in dogs whose livers had previously been injured by chloroform. Pratt[12] later found a

close correlation between the functional activity of the liver as determined by the bromsulfalein test and the duration of action of pentobarbital sodium. Carbon tetrachloride was used to produce hepatic injury. The action of barbital sodium, however, was not increased when administered to a dog with a damaged liver. He concluded that the short-acting pentobarbital sodium was rapidly destroyed by the liver, whereas barbital sodium was more stable and was not detoxified. This was supported by the fact that barbital sodium is largely excreted unchanged in the urine.

Cameron and de Saram[3] found that the duration of anesthesia produced by pentobarbital sodium and evipan was more than doubled following liver injury produced by carbon tetrachloride in rats. Acute liver damage did not significantly affect the action of barbital sodium or phenobarbital.

Neither of the hepatotoxins, chloroform or carbon tetrachloride, which were used in the foregoing studies, is specific in its action since, in addition to producing hepatic necrosis, they result in a certain amount of generalized tissue injury.[2,5,9] Koppanyi[9] had this in mind when he advanced a hypothesis to explain the increased potency exhibited by certain barbiturates in the presence of liver damage. He suggested that the hepatotoxin, chloroform, injured the central nervous system so that the nerve cells became more susceptible to the barbiturates. He further suggested that the general depression in cellular metabolism resulting from the hepatotoxin might be a factor in increasing the potency of the barbiturates following chemical injury of the liver. A method which would eliminate a constant amount of functioning hepatic tissue without introducing other unmeasurable factors would be desirable. Such a method is found in partial hepatectomy.

One of us (Higgins) and Anderson[7] found that restoration of the liver following partial hepatectomy in the rat began toward the end of the first 24 hours. The remnant more than doubled itself in 72 hours and by 10 to 14 days the ratio of liver weight to body weight was restored to normal.

It was thought that if the liver actually did protect against the action of certain drugs, then removal of a portion of this organ should increase the potency of these substances. Similarly, restoration of the liver should be accompanied by a corresponding decrease in the effects of their action.

In our experiments three drugs of the hypnotic-anesthetic group were tested, namely ethyl-o-ethylphenylurea,* a hypnotic of low toxicity,[8] pentobarbital sodium (nembutal) and pentothal sodium.† Ethyl-o-ethylphenylurea was administered intraperitoneally to rats in doses of 100 mg. per kg. The barbiturates were given intra-

* Supplied by Burroughs Wellcome & Co., Inc., through the courtesy of Dr. R. C. Page. Not released for clinical use.

† Supplied through the courtesy of the Abbott Laboratories.

venously,[14] pentobarbital sodium in 1% aqueous solution and pentothal sodium in 2% solution. The doses were 25 and 30 mg. per kg. respectively. The duration of action of these substances was taken as the interval during which the rats would lie quietly on their backs or sides. The rats were tested frequently to make sure that physiologic sleep was not superimposed on the induced sleep.

Over a period of five trials in which ethyl-o-ethylphenylurea was given, the duration of anesthesia averaged 42 minutes in a group of 8 rats (Chart 1). Then partial hepatectomy was carried out. Seventy per cent of the liver was removed by this procedure. Two days later the drug was again administered. At this time the duration of

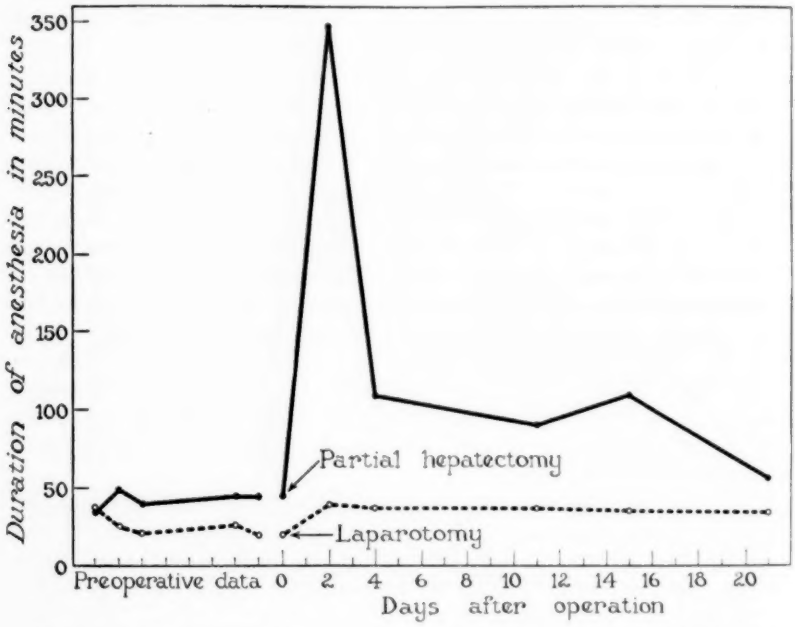

CHART 1.—Effect of partial hepatectomy on the action of ethyl-o-ethylphenylurea.

anesthesia had increased to 347 minutes or about eight times the preoperative level. On the fourth postoperative day when the liver was more than half restored, the duration of anesthesia had decreased to 118 minutes, still about three times the preoperative level. Thereafter it decreased but tended to lag behind the corresponding degree of hepatic restoration.

In the control series the drug was given at corresponding intervals but instead of partial hepatectomy, laparotomy was done. There was only a slight prolongation of anesthesia following laparotomy.

In a series of 7 rats, pentobarbital sodium was given every 2 days for a period of 5 trials. It was noted that for each rat a degree of

tolerance was developed after the first dose,[11] but in the remaining 4 trials the duration of anesthesia remained around 50 ± 5 minutes (Chart 2). On the second day following partial hepatectomy the duration of anesthesia had increased to 212 minutes or four times the preoperative level. Thereafter it decreased in an irregular but definite manner until by the fourteenth postoperative day it was within the preoperative range.

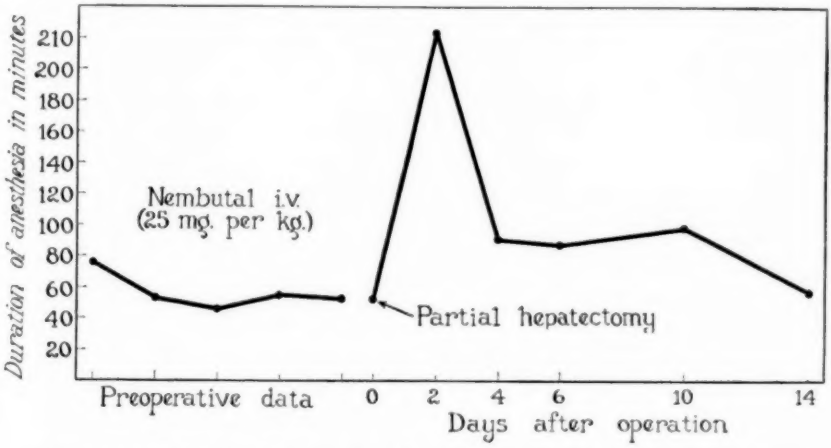

CHART 2.—Effect of partial hepatectomy on the action of pentobarbital sodium.

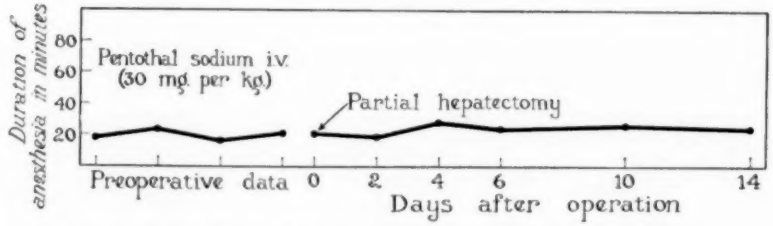

CHART 3.—Effect of partial hepatectomy on the action of pentothal sodium.

The action of pentothal sodium was studied in 9 rats, using the same technique. Partial hepatectomy had no significant effect on its action (Chart 3).

Unilateral nephrectomies were done on 6 rats to serve as controls. This procedure had no effect on the action of either pentobarbital sodium or pentothal sodium.

Comment. It has frequently been stated that the short-acting barbiturates are unstable and so are quickly destroyed in the body, presumably by the liver, whereas those of longer action, being more stable, resist detoxication and are largely excreted in the urine.[1,12,14] Pentobarbital sodium is one of these short-acting barbiturates.

The results of this study show that its action is increased by a reduction in the amount of functioning hepatic tissue, thus suggesting destruction by the liver. Pentothal sodium is even more short-acting than pentobarbital sodium and is its sulphur-containing homologue. Thus, it was expected that partial hepatectomy would prolong the action of pentothal sodium even more than that of pentobarbital sodium. This did not occur. Although several writers have attributed its destruction to the liver,[6,10] these data suggest that the liver is little concerned with the destruction of pentothal sodium. Clinical support of this observation is found in Adams' statement that "it has been observed clinically that patients who have hepatic damage appear to require as much of the drug to produce narcosis as do those patients who have no hepatic damage."[4]

As for ethyl-o-ethylphenylurea, this study simply shows that its action is augmented by a reduction in the amount of functioning hepatic tissue.

The method of partial hepatectomy has eliminated both the possibility of induced nerve cell hypersensitivity and generalized tissue injury from the interpretation of results, thus defining more clearly the protective function of the liver.

Summary and Conclusions. The duration of anesthesia in rats produced by ethyl-o-ethylphenylurea and pentobarbital sodium was markedly prolonged following partial hepatectomy. This procedure had no effect on the action of pentothal sodium. Unilateral nephrectomy did not prolong the action of either pentobarbital sodium or pentothal sodium. These data suggest that the liver is instrumental in protecting the animal against the action of ethyl-o-ethylphenylurea and pentobarbital sodium but does not protect against the action of pentothal sodium.

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BOOK REVIEWS AND NOTICES

OBSTETRICS AND GYNECOLOGY. In two volumes. By the Departmental Staff of The University of Chicago and Other Contributors. Edited by FRED L. ADAIR, M.A., M.D., F.A.C.S., Mary Campau Ryerson Professor and Chairman of the Department of Obstetrics and Gynecology in The University of Chicago; Chief of Service, The Chicago Lying-in Hospital, Chicago. Pp. Vol. 1, 1000; Vol. 2, 1031; illustrations Vol. 1, 359 and 14 plates; Vol. 2, 304 and 10 plates (plates mostly in color). Philadelphia: Lea & Febiger, 1940. Price, $20.00.

THERE has been an increasing tendency in recent years toward combining obstetrics and gynecology for the purposes of practice, teaching and research. This fact is being recognized more and more by the publication of such combined books as the present ones.

These two volumes take up their subjects in the following order: 1, Basic biologic relationships of the human female. 2, Approach to individual and communal problems. 3, Life cycle of the human female. 4, Normal and abnormal conditions of the non-pregnant and pregnant woman. 5, Normal and abnormal conditions of the parturient woman. 6, Normal and abnormal conditions of the postpartum woman and of the newborn infant. 7, Diseases of the female genitalia. 8, Medical and surgical specialties in relation to obstetrics and gynecology. 9, Obstetric and gynecologic operative procedure.

The authors have devoted more space to obstetrics than to gynecology, in which selection the Reviewer heartily agrees. They present the point of view of their own clinic, rather than give many points of view upon subjects which are still in a state of flux. They purposely have omitted a detailed treatment of many technical matters in order to give more emphasis to principles than practice. Many subjects not heretofore covered in textbooks of gynecology and obstetrics receive careful and interesting treatment. All topics are considered from a very sane and broad point of view. The Reviewer is impressed chiefly by the wide variety of subjects touched upon and the large amount of very interesting material which has been brought together in one set of books. Both volumes make very interesting reading. They would seem to be excellent additions to the library of the general practitioner as well as the specialist. The undergraduate, however, usually demands a short cut to knowledge, which means volumes of small size.

D. M.

THE MEDICAL CAREER AND OTHER PAPERS. By HARVEY CUSHING. Pp. 302. Boston: Little, Brown & Co., 1940. Price, $2.50.

THE 16 addresses and biographical sketches that comprise this volume were selected for publication by the author in the summer before his death. As explained by Dr. Fulton in the Preface, it forms a companion volume to *Consecratio Medici*, all but 3 of the collection having appeared since publication of the latter in 1928. The 7 essays are all "occasional," mostly presidential addresses, ranging from advice to Dartmouth students ("The Medical Career"), through consideration of medical societies, schools and libraries to one of the author's own specialty ("Psychiatrists, Neurologists and Neurosurgeons"). Beaumont, the Drs. Welch, Halsted, James Ford

Rhodes, George Derby, Perry Williams Harvey, Haller, Councilman and the Mayo brothers are the subjects of the biographies. Authors of lesser ability might easily be hampered by subject restrictions, in the composition of such essays, necessarily carried out amid the distractions of an active surgical practice. But not so Dr. Cushing! As one should expect from his previous literary efforts, he has woven into his presentations a pleasing combination of practical common sense and humor—now and then with an edge to it—of historical knowledge and shrewd insight into present and probable future trends. The essays disclose an able man of letters and illustrate what may be got from thoughtful and understanding study of medical history.

E. K.

POISONS. THEIR ISOLATION AND IDENTIFICATION. By FRANK BAMFORD, B.Sc., Late Director of the Medico-Legal Laboratory, Cairo. With a Foreword by PROF. SYDNEY SMITH, M.D., F.R.C.P., Regius Professor of Forensic Medicine, University of Edinburgh. Pp. 344; 21 illustrations. Philadelphia: The Blakiston Company, 1940. Price, $4.00.

THIS book can be recommended as a practical laboratory manual for chemists engaged in the detection of poisons. The material, concisely presented, credibly covers the field of Forensic Chemistry. Chapter X contains a qualitative analytical scheme for the identification of the alkaloids and will be especially welcomed by toxicologists.

F. S.

ARTIFICIAL PNEUMOTHORAX. Its Practical Application in the Treatment of Pulmonary Tuberculosis. Contributions by Saranac Lake Physicians to the Studies of the Trudeau Foundation. Editorial Committee: E. N. PACKARD, M.D., J. N. HAYES, M.D., and S. F. BLANCHET, M.D. Foreword by E. R. BALDWIN, M.D. Pp. 300; 85 illustrations and 33 tables. Philadelphia: Lea & Febiger, 1940. Price, $4.00.

SEVENTEEN physicians of the Saranac Lake group have contributed chapters to this compact monograph. Intended as a practical handbook, the volume is simple and comprehensive. The selection of cases, choice of apparatus, technique of operation, and management of treatment are discussed. Detailed consideration is given to the numerous complications of pneumothorax therapy. The place of artificial pneumothorax in the treatment of tuberculosis is sanely evaluated. Those experienced in pneumothorax will find the book relatively elementary. Those gathering their early experience in this form of therapy, on the other hand, will find it very useful as a guide.

H. I.

THE BRITISH ENCYCLOPÆDIA OF MEDICAL PRACTICE including Medicine, Surgery, Obstetrics, Gynæcology, and Other Special Subjects. *Cumulative Supplement, 1939* (pp. 170; 1 illustration); *Surveys and Abstracts, 1939* (pp. 655; 26 illustrations). Under the General Editorship of SIR HUMPHRY ROLLESTON, BT., G.C.V.O., K.C.B., M.D., D.Sc., D.C.L., LL.D., Emeritus Regius Professor of Physic, Cambridge; Sometime President of the Royal College of Physicians of London. Publishing Editor, ADAM CLARK, L.M.S.S.A., Sub-Editor, G. FAULKNER, D.Sc. London: Butterworth & Co. (Publishers), Ltd., 1940. Price, $9.00 per volume.

THIS encyclopedia now publishes 2 volumes such as these annually. The large supplementary volume of Surveys and Abstracts for 1939 is chiefly devoted to abstracts of current medical literature arranged alphabetically by subject, with frequent references to the original treatment of the subject in earlier volumes. The subjects, which range from abdominal pain to yellow fever, are not listed separately, though of course all appear

in the index. This section, like similar abstract annuals, is useful in offering the reader a critical selection from the vast amount of published work. The earlier portion of the book contains "critical surveys" of 27 topics, some covering whole specialties, others such items as Q fever and contact lenses, with 2 surveys on drugs.

The smaller Cumulative Supplement, following the same alphabetical method as the former volume, aims to present new and generally accepted medical knowledge, replacing the presentation of the original volume and in turn to be replaced by the volume of the next year. It must be used "in conjunction with the original volumes of the Encyclopedia," and with the Surveys and Abstracts to which frequent reference is made. This seems like a cumbersome method, its only advantage over the loose-leaf systems being that there are no loose leaves to be inserted. E. K.

DIAGNOSIS AND TREATMENT OF HEAD INJURIES. By SIDNEY W. GROSS, M.D., F.A.C.S., Attending Neurosurgeon, Beth Israel Hospital; Associate Neurosurgeon, Morrisiania City Hospital; Adjunct Neurosurgeon, Mt. Sinai Hospital, etc., and WILLIAM EHRLICH, M.D., Associate Attending Neurosurgeon, Newark Beth Israel Hospital; Neurosurgeon, Barnett Memorial Hospital, Paterson, etc. Introduction by PERCIVAL BAILEY, M.D., PH.D., Professor of Neurology and Neurosurgey, University of Illinois, Chicago. Pp. 275; 94 illustrations. New York: Paul B. Hoeber, Inc., 1940. Price, $5.00.

THIS is a well-written, close-knit book that supplies adequate information on the diagnosis and treatment of head injuries without introducing controversial or non-essential material. The chapters on anatomy, physiology and pathology of cranial trauma are short and concise but cover all important details. Methods of examination are briefly outlined and their relative values stressed. In discussing the classification of head injuries any lengthy discussion as to the nature of concussion has been wisely avoided. The chapter on treatment is well thought out, the dangers of dehydration and indications for and against lumbar puncture clearly indicated, with emphasis on rest as the best means of handling these cases.

In discussing the management of compound fractures the methods used in handling cases which are complicated by dural tears and a cerebrospinal fluid leak are properly described in greater detail. The clinical picture accompanying extradural and chronic and acute subdural hemorrhage is adequately discussed and the operative treatment, a sub-temporal decompression for extradural, and bilateral trephines or a small bone flap for subdural clots given concisely.

In the chapter on skull fracture involving the sinus greater emphasis might perhaps have been laid on the dangers of irrigating the external canal of an ear through which cerebrospinal fluid is escaping. Meningitis, brain abscess and osteomyelitis together with the treatment of these complications are briefly discussed.

An adequate description of the proper methods of handling gunshot wounds of the head based for the most part on Cushing's technique developed in the first World War is of timely interest. A chapter is devoted to a description of the armamentarium necessary for the surgical procedures demanded by cranial traumatic cases. Finally the sequelæ consequent upon head injury are discussed. It seems unfortunate that emphasis has been placed upon the relationship between cranial trauma and brain tumor, although in discussing the medico-legal aspects of post-traumatic sequelæ this subject could not have been avoided.

This book can be recommended for students and general practitioners especially as giving a straight-forward conservative description of the

methods best established for the treatment of cranial trauma. Incidentally the introduction by Percival Bailey affords an excellent text around which to write a book on this subject as he outlines very clearly the general principles of such management.

The format is good, the type large and pleasing to the eye, the illustrations adequate and pertinent to the context. F. G.

THE MARCH OF MEDICINE. Edited by the Committee on Lectures to the Laity of the New York Academy of Medicine, WILLIAM C. WHITE, Chairman. Pp. 168; 1 illustration. New York: Columbia University Press, 1940. Price, $2.00.

IN this volume are presented 6 of the 7 lectures comprising the fourth series of the New York Academy of Medicine's lectures to the laity. Dr. Walter C. Alvarez, in "From Folkways to Modern Medicine," illustrates the opposing methods and purposes of the regular and irregular practitioners from the earliest times to the present. Dr. Sanford V. Larkey's "Health in Elizabethan England" contains an immense amount of interesting and thought-provoking information concerning the public health in England's "Golden Age." In "Not So Long Ago," Dr. Cecil K. Drinker warms over entertainingly some of the material that appeared in his book of the same name based on the fascinating diary of his great-great-great grandmother, Elizabeth Drinker, of Philadelphia. Dr. Charles Gordon Heyd, in "The Romance of Modern Surgery," by a number of references to early surgical practices points up the boon conferred on humanity by modern discoveries and procedures beginning with ether anaesthesia and "Listerism." Dr. R. G. Hoskins' "Story of Insanity" reviews reforms in the treatment of the insane made possible by gradually more enlightened concepts of the nature of insanity. Dr. Karl A. Menninger rather surprisingly takes before a possibly bewildered public the Case of the Rest of the Medical Profession *vs.* Psychiatry ("The Cinderella of Modern Medicine"), presenting evidence favorable to both sides of the controversy. The volume contains an index.

These rather informal lectures should, as a whole, make pleasant, casual reading for a not-too-critical public, but (with one exception) may possibly leave the more thoughtful layman with a feeling that the constructive values of the opportunity offered have not been fully realized. The full potential value of medical lectures to the laity is as obvious as it is difficult to achieve. One wonders if, in time, such series of lectures will not be as systematically planned and integrated, and composed with as rigorous attention to a desired educational end, as are the best medical courses for students of medicine. W. McD.

CHEMOTHERAPY AND SERUM THERAPY OF PNEUMONIA. By FREDERICK T. LORD, M.D., Clinical Professor of Medicine, Emeritus, Harvard Medical School; Member of the Board of Consultation, Massachusetts General Hospital, ELLIOTT S. ROBINSON, M.D., PH.D., Director, Division of Biologic Laboratories, Massachusetts Department of Public Health, and RODERICK HEFFRON, M.D., Medical Association, The Commonwealth Fund; formerly Field Director, Pneumonia Study and Service, Massachusetts Department of Public Health. Pp. 174. New York: The Commonwealth Fund, 1940. Price, $1.00.

THE authors have presented an accurate, authoritative and comprehensive book on the treatment of pneumonia. This book represents the first complete review of chemotherapy in pneumonia and also brings up to date the use of anti-pneumococci serum. The keynote is found in the first sentence: "Pneumonia is a medical emergency." Throughout this volume the authors

stress the importance of an early and accurate diagnosis. Fifty-eight pages are devoted entirely to the use of serum, whereas treatment with sulfapyridine occupies 25 pages. This should in no way be interpreted as indicating the relative importance of the two forms of therapy. Every practicing physician would profit by reading this entire book as it contains practical suggestions pertaining to the intelligent handling of pneumonia patients.

H. F.

NEW BOOKS

Pathogenic Anærobic Organisms of the Actinomyces Group. By DAGNY ERIKSON. (Special Report Series, No. 240 of Medical Research Council.) Pp. 63; illustrated. London: His Majesty's Stationery Office, 1940. Price, 1s. 0d.

The New International Clinics, Vol. 2 (N.S. 3), 1940. Edited by GEORGE MORRIS PIERSOL, M.D., Professor of Medicine, Graduate School of Medicine, University of Pennsylvania, Philadelphia. With 17 Collaborators. Pp. 365; illustrated. Philadelphia: J. B. Lippincott Company, 1940.

The review deals with present concepts of the hypothalamus.

St. Thomas's Hospital Reports. Second Series, Vol. IV. Editors: PROF. O. L. V. S. DE WESSELOW, MR. C. MAX PAGE, assisted by MR. N. R. BARRET, DR. J. ST. C. ELKINGTON, DR. A. J. WRIGLEY. Pp. 198; illustrated. London: St. Thomas's Hospital, 1939. Price, 10s.

The Medical Clinics of North America, May, 1940 (Vol. 24, No. 3, New York Number). Pp. 362; 41 illustrations. Philadelphia: W. B. Saunders Company, 1940.

This New York number contains 29 articles on the usual wide diversity of subjects.

Complete Guide for the Deafened (pp. 256) and *Handbook of Hearing Aids* (pp. 156). By A. F. NIEMOELLER, A.B., M.A., B.S. With Forewords by HAROLD HAYS, M.D., F.A.C.S. New York: Harvest House, 1940. Price, $3.00 each.

The Varieties of Human Physique. An Introduction to Constitutional Psychology. By W. H. SHELDON, PH.D., M.D., Harvard University. With the Collaboration of S. S. STEVENS, PH.D., Harvard University, and W. B. TUCKER, M.D., University of Chicago. Pp. 347; 104 illustrations. New York: Harper & Brothers Publishers, 1940. Price, $4.50.

A Manual of Otology, Rhinology and Laryngology. By HOWARD CHARLES BALLENGER, M.D., F.A.C.S., Assistant Professor of Otolaryngology, Northwestern University School of Medicine, Chicago. Pp. 302; 90 illustrations and 4 color plates. Philadelphia: Lea & Febiger, 1940. Price, $3.75.

NEW EDITIONS

Neoplastic Diseases. A Treatise on Tumors. By JAMES EWING, A.M., M.D., Sc.D., LL.D., Professor of Oncology at Cornell University Medical College, New York City; Consulting Pathologist, Memorial Hospital. Pp. 1160; 581 illustrations. Fourth Edition, revised and enlarged. Philadelphia: W. B. Saunders Company, 1940. Price, $14.00.

Physical Therapy for Nurses. By RICHARD KOVÁCS, M.D., Clinical Professor and Director of Physical Therapy, New York Polyclinic Medical School and Hospital; Attending Physical Therapist, Manhattan State, Harlem Valley State and West Side Hospitals, etc. Pp. 335; 99 illustrations. Second Edition, thoroughly revised. Philadelphia: Lea & Febiger, 1940. Price, $3.25.

Principles of Hematology. By RUSSELL L. HADEN, M.A., M.D., Chief of the Medical Division of the Cleveland Clinic, Cleveland; Formerly Professor of Experimental Medicine in the University of Kansas School of Medicine, Kansas City, Kansas. Pp. 362; 104 illustrative cases, and 167 illustrations including 173 original photomicrographs and 100 original charts and drawings. Second Edition, thoroughly revised. Philadelphia: Lea & Febiger, 1940. Price, $4.50.

The Diagnosis and Treatment of Pulmonary Tuberculosis. By JOHN B. HAWES, 2d, M.D., Late President of the Boston Tuberculosis Association; Director of the National Tuberculosis Association, etc., and MOSES J. STONE, M.D., Assistant Professor of Medicine, Boston University, School of Medicine; Physician to the Chest Clinic of the Massachusetts Memorial Hospital, Boston, etc. Pp. 260; 75 illustrations. Second Edition, revised by DR. MOSES J. STONE, with a Foreword by RICHARD C. CABOT, M.D. Philadelphia: Lea & Febiger, 1940. Price, $2.75.

An Introduction to Biochemistry. By WILLIAM ROBERT FEARON, M.A., Sc.D., M.B., F.I.C., Fellow of Trinity College, Dublin; Member of the Royal Irish Academy. Pp. 475. Second Edition. St. Louis: The C. V. Mosby Company, 1940. Price, $3.75.

A Textbook of Physiology. By WILLIAM H. HOWELL, PH.D., M.D., Sc.D., LL.D., Emeritus Professor of Physiology in The Johns Hopkins University, Baltimore. Pp. 1117; 330 illustrations. Fourteenth Edition, thoroughly revised. Philadelphia: W. B. Saunders Company, 1940. Price, $7.50.

Textbook of Biochemistry. By BENJAMIN HARROW, PH.D., Professor of Chemistry, City College, College of the City of New York. Pp. 439; 88 illustrations. Second Edition, revised. Philadelphia: W. B. Saunders Company, 1940. Price, $3.75.

PROGRESS
OF
MEDICAL SCIENCE

SURGERY.

UNDER THE CHARGE OF
I. S. RAVDIN, B.S., M.D.,
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SOME OBSERVATIONS ON PERFORATED GASTRIC AND DUODENAL ULCER.

THE mortality from perforated peptic ulcer is still high, despite the advances which have been made in the general postoperative care of patients. Factors which influence the mortality rate in this not uncommon catastrophe are at times difficult to analyze, despite the fact that there is more or less agreement that peritonitis or intra-abdominal abscess is the most usual cause of death.[8,11,22,29a] Most of the published reports are concerned chiefly with benign ulcers which perforate into the free abdominal cavity. Obviously, ulcers which perforate into an area of preformed adhesions, posteriorly into the pancreas, or some other viscus, or preformations of malignant ulcers, present problems which are different from acute perforations of ulcers of the free portions of the duodenum or stomach in which widespread contamination of the peritoneum results.

The reported mortality statistics relating to this latter type are variable and reasons for the variations are not yet clear. Roscoe Graham[13a] reported a mortality of 3% in a series of 60 cases and intimated that the important factor in obtaining such excellent results was the fact that only closure of the perforation was done. Judin[20] reported a mortality rate of 12.8% in 418 cases operated upon for perforated peptic ulcer and suggested that resection of the stomach played an important rôle in attaining this low mortality, since in his experience the mortality decreased steadily as the number of resections increased. Junghanns[21] reported statistics from Schmieden's Clinic which indicated a steadily decreasing mortality in the period from 1914 to 1938. In the period from 1928 to 1938 there were but 3 deaths in 63 cases in which resection of the stomach was performed, a mortality of 4.8%. In the same period 52 cases were treated by simple suture of the ulcer with or without gastro-enterostomy, with 19 deaths, a mortality of 36.5%. While

Junghanns was not willing to ascribe the decrease in mortality solely to the type of operation which was performed, pointing out that in the more recent series the cases were operated upon earlier, he believed that the increased frequency of resections has played a definite rôle. Obviously, here are two diametrically opposed viewpoints regarding the best method of handling free perforations of peptic ulcers, one stating that the simplest procedure in the acute emergency is best and that time ought be taken to prepare the patient for operation, the other that a radical procedure should be performed as early as possible.

Despite the fact that reported statistics of perforated ulcer treated by immediate partial gastrectomy, since the paper by von Haberer,[15] in 1922, indicate mortality rates which are at least as good as those from simple closure, there is well-founded skepticism regarding the advisability of performing a procedure of such proportions in the presence of widespread peritoneal contamination. Since peritonitis offers the threat of major importance to life in these cases it does not seem logical to assume that gastrectomy will decrease its severity, or the possibility of a fatal outcome. It is logical, we believe, to assume that in addition to the deleterious effects of spill from the stomach or duodenum through the perforation, the increased mortality of partial gastrectomy is added and, therefore, the immediate mortality should be higher. Granting that secondary operations may be prevented if partial gastrectomy is performed, the effect on the immediate mortality is of major importance. Graham[13a] has said, "The responsibility of the surgeon is to save life." This object, we agree, can best be obtained by conservative surgical procedures. The advocates of resection in acute perforations[15,20,21,34] stress the importance of the greater relief from ulcer symptoms, following the radical operation, but it is well to note that the necessity for secondary operation following simple closure is not frequent,[10,22,23,30] although the recurrence of ulcer symptoms is not unusual.[28]

The treatment of uncomplicated duodenal ulcer has changed during the past 20 years, especially as regards the concept that duodenal ulcer was predominantly a surgical condition and required some form of operative procedure. Surgical intervention, however, should be reserved for those cases which do not respond to well-controlled medical therapy or in which sequelæ, such as perforation, obstruction, or massive or recurrent hemorrhage are present. Accordingly, following simple closure of a perforated ulcer subsequent surgical therapy should be based upon the same indications.

The method for handling cases of perforated ulcer must be based upon considerations more fundamental than variations in operative approach. The majority of studies on this problem[6,12,21,23,25,29b,31] stress the influence of the amount of time elapsing between perforation and operation. Variations in response to therapy in individual cases indicate that the time interval is not all important, since many patients who were otherwise in good condition die even when operated upon early, while others survive late operation, or even no operation at all. In this regard we have had 3 cases with an unquestioned rupture of a duodenal ulcer, with free air beneath the diaphragm, all of whom refused operation and recovered with no other therapy than supportive measures and constant suction drainage to the stomach. Nagel[26] also

reported a case treated by this method but it is obviously not the therapy of choice. Actually the factor of time is not the sole one. The reaction of the patient to the perforation and the total amount of material spilled are of nearly equal importance. That there is an increase in mortality with increasing period between perforation and operation has been frequently stressed. Time alone is not important, but rather what is happening during the time from perforation to operation.

The patient who drinks large quantities of fluid, whose stomach is lavaged, or who retches a great deal, is more likely to increase the amount of insult to his peritoneum than one who lies quietly, and has an empty stomach at the time of perforation. We have analyzed our own cases, 56 for a period of 1 year, and have noted that while there was the expected tendency toward higher mortality in the late cases, this was not constant, there being a greater mortality in the 4 to 8 hour period than in the 8 to 12 hour period.

The length of time from perforation should not alone be used for prognosis, nor for the decision as to operation in late cases. However, the concept that delay in instituting therapy is deleterious has played an important rôle in the decreased mortality rates. The prevention of delay in therapy is usually not difficult, since the patient as a rule seeks medical aid early. The diagnosis with few exceptions ought likewise be made early, since the onset of symptoms is sudden.

Perforation of a peptic ulcer is one of the most dramatic of incidents and the diagnosis is hardly to be missed. So sudden is the onset that the patient can usually tell the exact time. The associated pain, usually located in the epigastrium, is so acute that the patient lies perfectly still, fearing to move. Rigidity comes on early and is boardlike, especially in the upper abdomen. Despite the excessive pain the patient usually shows no sign of shock except pallor, the pulse and blood pressure as a rule remaining within normal limits. The diagnostic features of perforated ulcer are well described by Moynihan[25] and are therefore not reviewed in detail here. This clear-cut picture is present in about 90 to 95% of cases.[22,28] Rarely the condition may be confused with coronary artery occlusion, pancreatitis, or appendicitis. Vomiting is not usual in ruptured ulcer, but does occur. The fact that the patient has had no previous history suggestive of ulcer ought not be considered unusual, as this not infrequently happens. The number of cases reported without previous ulcer symptoms varies from 5 to 45%.[14] Moynihan[25] has stressed the fact that true surgical shock is not a part of the early picture and should not be expected. Several authors[4,5,33a] have stressed shock as a frequent accompaniment of acute perforations of peptic ulcer either by inference or direct statement. While the patient usually appears "hard hit" and might because of the nature of the accident be expected to be in true surgical shock, blood pressure levels are usually well maintained because of profound vasoconstriction. In our own experience we have rarely seen a picture simulating surgical shock until late when it is ascribable to a fatal peritonitis. This likewise has been the experience of others.[8,22,25]

It has been the general impression that perforated duodenal ulcer constitutes one of the few remaining surgical emergencies which require immediate operation. The few cases in which we have been forced,

because of the patient's condition, to delay operation has suggested to us that when these patients require pre-operative preparation, a few hours' delay may be well spent. This has been well stressed by Graham.[13b] Frequently the ulcer patient is in poor nutritive state.[2,17b,19] Recently the nutritive state of these patients has received attention, not only with relation to healing of the ulcer, but the healing of the wound after operation.[17a,b,32] The operative wound in these cases is prone to become infected or to disrupt. It has been pointed out that difficulty of wound healing in these cases may be associated with the same factors which caused the ulcer to perforate. There is no good reason why ulcers should perforate under ordinary conditions, and there is a suggestion that the perforation might well be due to general nutritive disturbances.[17b,19]

Even in the absence of the usual signs of shock, adequate fluids, blood or plasma should be administered before operation, unless the patient appears to be in very good condition. Emptying of the stomach is of value, provided this can be done without causing excessive retching. As a rule, this can be accomplished by a Levine tube passed through the nose. Occasionally this is effective in decreasing the amount of intraperitoneal spill from the stomach, but it must be remembered that in many cases the patient will have solid particles in the stomach which makes aspiration difficult. No attempt should be made to wash the stomach by the introduction of fluid through the tube because of the possibility of increasing the spill. We have not found it necessary to give soda bicarbonate or Seidlitz powders to increase the subdiaphramatic gas for diagnosis. The time necessary for preparing the patient for operation need not be long. Preparations should be started as soon as the patient presents himself for treatment and the first step should be centered about keeping the stomach empty, the patient quiet, and the restoration of fluid and salt balance.

The operation should be as simple and as non-traumatic as possible. Graham[13b] stressed the fact that extensive procedures are not necessary to effect a closure of the ulcer and that suturing omentum over the opening is all that was necessary. We prefer closure of the perforation with the sutures left long tying a piece of omentum over the opening. We see no need of extensive exploration in these cases and agree with most authors that the least done at the time of the operation is in the end best. Aspiration of as much of the intra-abdominal content as is easily accessible is of value. We can see no reason for routine drainage of these cases, although this is advocated by some authors.[9,11,24] It is quite impossible to drain the peritoneal cavity, and drains inserted may have the tendency to add additional insult to the peritoneum by providing access of bacteria and through direct trauma. There is no doubt that organisms are present in the peritoneal content of cases with free rupture of a duodenal or gastric ulcer.[7,8,11] Where there is a large amount of spill their dilution may be great enough to account for many of the negative cultures that are obtained. It has been stated that the gastric hydrochloric acid is sufficiently bacteriostatic to prevent growth.[33b] That acidity is not the sole factor is suggested by the fact that fluid removed from these cases, even soon after perforation, has a hydrogen-ion concentration approximating the blood plasma.[18] Furthermore, in a series of 20 such cases studied at the

Detroit Receiving Hospital by Dr. Charles M. Henry, the hydrogen-ion concentration of the stomach content at the time of operation was uniformly high, the chloride content low, and this state of affairs has been found to exist for several days after operation.

The operative procedure is greatly simplified in the case of perforated duodenal ulcer by the use of the incision described by Amendola[1] and Hartzell and Sorock.[16] Not only is trauma to the peritoneum minimized but the small transverse incision permits better pulmonary ventilation after operation, reduces the incidence of eviscerations and results in shorter convalescence. Patients operated upon through the incision described by Hartzell and Sorock[16] are able to be discharged after a week, so far as integrity of the wound is concerned.

Since intra-abdominal infection is the most common cause of death in these cases it may be suggested that the introduction of sulphanilamide into the peritoneal cavity, or by hypodermoclysis, as reported by Ravdin, Rhoads and Lockwood[27] for cases of ruptured appendicitis, would be of value. We have not used this procedure routinely in the case of ruptured peptic ulcer, as the incidence of frank infection is not so high as in the cases of perforation of the appendix. In cases operated upon late we feel the procedure has merit.

We prefer to perform the operation with spinal anesthesia because of the relaxation and quietness it affords to the abdominal wall and the viscera. Several authors agree with this choice of anesthetic.[11,13b,22] We have not noted that spinal anesthesia causes any increase in the spill of gastro-intestinal fluid. When we have introduced methylene blue into the stomach to outline the perforation[3] we have frequently found that the ulcer site was not colored. Surely if there was a tendency for the extrusion of additional material after spinal anesthesia, the methylene blue ought to leak out freely. We have not felt that the method is of great value, since the perforation is usually not difficult to find.

The perforation of a peptic ulcer is a complication which is in itself but an incident in the general problem of the ulcer patient. It demands early surgical intervention and because of the possibility of peritonitis resulting from such an accident it appears that the less trauma inflicted at the time of operation, the better. Following operation the treatment is similar to that in cases where peritonitis is imminent. Maintenance of fluid and salt balance, continuous decompression of the stomach, bed rest, and plasma or blood transfusions are important. Following recovery from the operative procedure it is well to bear in mind that the patient must be treated as an ulcer patient. While the perforation of a duodenal or gastric ulcer usually has a salutory effect on the patient as regards the manner in which he follows his diet, these patients ought to be considered as the usual ulcer patient and therefore subject to the difficulties incident to the disease. Subsequent surgical procedures ought to be based on the merits of the individual case, without particular reference to whether or not there was a previous perforation.

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OPHTHALMOLOGY

UNDER THE CHARGE OF

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THE PATHOGENESIS OF SYPHILITIC OPTIC ATROPHY.

In a series of recent papers, Moore and his coworkers[2–4] have reviewed the prevailing conceptions of the nature of the involvement of the optic nerves in syphilis of the central nervous system. They have discussed the pathology and probable pathogenesis of so-called primary optic atrophy and have considered the effects on the progress of the atrophy of modern as compared to older methods of treatment. Though none of the problems connected with this disease can be considered to be finally solved, yet considerable interesting and valuable data have been accumulated.

Syphilis of the central nervous system may involve the optic nerves in three distinct ways. There may be primarily an optic neuritis which may subside without residuals or may result in secondary optic atrophy. There may be an axial or retrobulbar neuritis, the primary expression of which is a central scotoma without visible changes in the optic nerve and the end-result retrobulbar atrophy. Finally, there may be simple (non-inflammatory), progressive, so-called primary atrophy. In the

combined series of Woods and Dunn and Woods and Rowland, quoted by Moore, 45 of 58 syphilitic lesions of the optic nerves (77.5%) were of this last type. Even within this group of simple atrophies, however, considerable clinical variations occur. In most of the cases, the changes in the visual fields are essentially concentric constrictions of varying form. But in some cases the changes in the fields are of bitemporal or homonymous type suggesting involvement of the chiasm or optic tracts. It has been suggested, however, that these latter types of field defects may represent sector-like involvements of the optic nerves proper, rather than actual involvement of the chiasm or tracts. Moore and his coworkers seem to be inclined to consider in a single group all cases of optic atrophy in which there is evidence of more or less diffuse involvement of the nerve and in which there is no ophthalmoscopic evidence of preceding inflammation.

Following the lead of Wilbrand and Saenger, many ophthalmologists have been inclined to believe that simple atrophy of the optic nerve might develop in connection with syphilis of the central nervous system in two rather distinct forms with rather characteristic differences in visual field changes, mode of progression, response to treatment, pathology and pathogenesis. In the first type, there occurred gradual diminution of central vision and progressive contraction of the fields for form and color, the color fields showing greater loss proportionately than the form fields. These changes were taken to indicate diffuse involvement of the nerve, the true tabetic optic atrophy. It was assumed that in these cases the underlying lesion was that suggested by Uhthoff,[5] a primary degeneration of the ganglion cells and nerve fiber layer in the retina which ascended along the optic pathway in a centripetal direction. Secondly, Wilbrand and Saenger described a type of case in which concentric contraction of the form fields occurred while central vision remained normal and color perception was good in the functioning portions of the field. It was assumed that in these cases the intracranial portion of the optic nerves was involved in association with a basal meningitis. In the true primary tabetic optic atrophy, treatment was regarded as useless and even harmful, and blindness was regarded as the inevitable ultimate result. In the cases of the second type, associated probably with basal meningitis, treatment was often successful in arresting the progress of the atrophy.

More recent studies of the pathologic histology of the optic nerves in central nervous system syphilis raise considerable doubt as to whether there is any such basic distinction between types of syphilitic simple optic atrophy. According to Epstein,[1] Léri stated in 1904 that he had found numerous intact ganglion cells in the retina in the presence of complete optic atrophy and that ganglion cells were constantly present, irrespective of the presence or absence of myelinated fibers in the optic nerve. In 1913, Stargardt, quoted by Moore and by Epstein, demonstrated that, in syphilitic simple optic atrophy, the earliest histologic changes were to be observed in the chiasm and intracranial portion of the optic nerve in the form of undue proliferation of the glia fibers in the pial and subpial zones of the nerve, with isolated plasma cells in the overlying pia. Along with increasing infiltration of round and plasma cells in the overlying pia and proliferation of glia fibers in the nerve, degeneration of the medullary sheaths underlying the

infiltrated areas occurred, to be followed later by degeneration of the axis cylinders. The nerve fibers were ultimately replaced by glia cells filled with amyloid. The intraneural connective tissue and the blood-vessels usually were not involved even in far-advanced cases.

Since the work of Stargardt, histologic studies of the optic nerve in syphilitic optic atrophy have been made by Palick-Szanto, Richter, Fujiwara, Behr and Igersheimer. These authors agree that the atrophic process starts in the intracranial portion of the optic nerve and in the marginal fibers of the nerve. Apparently Richter and Palick-Szanto believe that the lesions of the nerve proper are secondary to inflammatory changes in the overlying meninges. Behr thinks that the primary change in syphilitic optic atrophy is an inflammatory process in the glial connective tissue septa of the intracranial portion of the nerve and of the blood-vessels that run in them. He emphasizes the effect of vascular changes on the nutrition of the nerve fibers and thinks the exudative changes in the meninges are coincidental. Igersheimer also is doubtful of the relationship of the degenerative to the inflammatory process. He states that round and plasma cell infiltration of the pia and arachnoid near and around the chiasm and the intracranial portion of the optic nerve is frequent, whether or not optic atrophy is present. He reports 1 case in which there was definite optic atrophy with only minimal cellular infiltration in the pia and none in the septa of the nerve. According to Moore, Fujiwara made histologic studies of the visual pathways in 19 cases of neurosyphilis, in only 2 of which optic atrophy had been demonstrated clinically. Cellular infiltration of the overlying pia was present in 17. Some degree of atrophy of the nerve fibers could be demonstrated in the intracranial portion of the optic nerve in 17, in the chiasm in 11, and in the tracts in 3.

With reference to the rôle played by the direct presence of the organisms in the tissues *versus* a toxin in the causation of optic atrophy, Moore quotes the work of Igersheimer, who searched for treponema in the optic pathways of 40 neurosyphilitics. In 1 of 10 cases in which the optic nerves were normal, a single organism was demonstrated in the optic tracts. Organisms were found in 3 of 9 cases in which there were marked inflammatory changes in the pia about the chiasm and nerves but no degeneration of the nerves, and in 7 of 21 cases in which optic atrophy was present. Experimentally, Igersheimer injected an emulsion of *Treponema pallidum* into the common carotid artery of 4 rabbits. In 3, extensive lesions developed in the choroid and retina with ascending atrophy of the optic nerve secondary to injury to the ganglion cells of the retina. In 1, a moderate degree of optic atrophy developed without marked intra-ocular changes.

In evaluating the work of these various authors, Moore concludes that it has been settled only that optic atrophy starts in the marginal fibers of the intracranial portion of the optic nerve distal to the chiasm. He states that, apparently, the atrophic process bears no direct relationship to the presence or absence of exudative changes in the overlying or neighboring meninges or to the presence or absence of *Treponema pallidum* in or close to the visual pathways.

The most recent report on the histologic changes in syphilitic optic atrophy has been made by Epstein (March, 1940). A man, aged 57, had been blind in the right eye for 20 years and had had progressive

loss of vision in the left eye for 3 months, due to bilateral optic atrophy. Neurologic evidences of tabes were present. The cerebrospinal fluid showed mildly positive findings consistent with a predominantly meningeal type of neurosyphilis. At necropsy the findings were those of chronic syphilitic meningitis rather than of general paresis or tabes. Both optic nerves were adherent to the dural sheath. The optic nerves, chiasm and tracts were extremely thin and were grayish on cross-section. The optic nerves and retinas were studied histologically. The meningeal infiltration seen elsewhere in the nervous system was present in considerable amount throughout the intracranial portion of the optic nerves, the chiasm and the tracts. In front of the orbital foramen it was slight and practically none was seen around the most anterior part of the optic nerves. In the chiasm the entering vessels were all surrounded by cuffs of lymphocytes, but anterior and posterior to the chiasm very few lymphocytes could be found within the parenchyma of the nerve, except in a narrow zone under the pia. Anterior to the optic foramen the subarachnoid space was obliterated and the pia and arachnoid were thickened and adherent in many places to the dura. The connective tissue septa were considerably thickened near the surface of the nerve, but those near the center were less affected. The pia and arachnoid covering the intracranial portion of the optic nerves and tracts were thicker than normal. There was no evident disease of the walls of the small vessels in the optic pathways beyond the infiltration with lymphocytes. In the right optic nerve, demyelinization was almost complete. A number of myelin sheaths could be seen in the most anterior portion of the nerve, but these disappeared farther back. Near the optic papilla they were associated with a much larger number of axis cylinders scattered fairly evenly through the core of the nerve but scanty near its surface. Most of these axis cylinders disappeared also in the region of the optic foramen. In the left optic nerve, demyelinization was greatest on the surface but there was a sector of destruction which penetrated deeper into the nerve. There was a considerable paucity of myelin sheaths even in the core of the nerve. Axis cylinders were more numerous than myelin sheaths and were present even in areas of complete demyelinization. Behind the chiasm the right optic tract contained many fewer myelinated fibers than the left. Both retinas showed great loss of nerve cells and fibers in the inner layer. In the right very few nerve cells remained; in the left a considerable number were preserved in the neighborhood of the macula. The granular layers and the rods and cones were well preserved everywhere. There was a moderate excess of neuroglial fibers in the nerve fiber layer. The optic nerves showed intense neuroglial sclerosis throughout their intra-orbital course.

In this case, Epstein summarizes, there was diffuse thickening of the leptomeninges with infiltration with lymphocytes. Lesions were present in the optic nerves, chiasm and tracts. There was good evidence of a fairly close relationship between the inflammatory and degenerative reactions in the nervous system. These observations suggest that chronic meningeal inflammation is the cause of optic atrophy. The position of the crossed nasal fibers on the meningeal surface of the optic tracts makes them more liable to be affected by inflammation. This may explain the tendency to bitemporal hemianopsia in certain cases.

It would seem, then, that in the opinions of most of the recent students of the pathologic anatomy of syphilitic optic atrophy the conception of the atrophy as a primary degenerative process is giving way to the idea that it is secondary to inflammation, either in the surrounding meninges or in the nerve itself. One objection to this concept of the primary influence of inflammation in the production of optic atrophy lies, however, in the frequency of occurrence of optic atrophy in the various forms of neurosyphilis as classified by Moore. In the early form of meningeal neurosyphilis, acute syphilitic meningitis or neurorecurrence, involvement of the optic nerve is frequent in the form of optic neuritis or neuroretinitis. This is supposed to subside without residuals, however. In late syphilitic meningitis, discrete or diffuse gummatous infiltration of the meninges, especially about the base, optic atrophy does occur at times, apparently. In meningovascular or cerebrospinal neurosyphilis, the occurrence of optic atrophy is questionable. In vascular neurosyphilis, the type characterized by cerebrovascular accidents, optic atrophy is uncommon. It is in parenchymatous neurosyphilis that optic atrophy is most frequently encountered. And it is worthy of note that optic atrophy occurs less frequently in pure paresis, in which organisms are readily demonstrable in the tissues in large number, than in pure tabes, in which organisms can be demonstrated rarely if at all. These facts have led to the concept that optic atrophy results from a toxic rather than an inflammatory affection of the nerve fibers.

In recent years, several new theories of the pathogenesis of syphilitic optic atrophy have been advanced and supported by various authors. These various theories have been critically considered by Moore and Woods in their most recent contribution on the subject of syphilitic optic atrophy. It is of interest to review these theories in the light of the criticisms proposed by Moore and Woods.

Abadie advanced the theory that optic atrophy developed as a result of a nutritional disturbance of the nerve due to syphilitic involvement of its blood supply. He noted that, in many cases of tabetic optic atrophy, the retinal arteries were constricted. He thought that this was due to a disturbance of the sympathetic nervous control of the central artery of the retina by a lesion in or near the ciliospinal center in the medulla similar to that causing the Argyll-Robertson pupil. The constriction of the vessels was believed to be progressive and permanent, thus leading to progressive ischemia of the retina and optic nerve and to ascending degeneration of the nerve. In the opinion of Moore and Woods, no proof of the validity of this theory has been advanced from a pathologic, clinical or therapeutic standpoint. The administration of atropine or acetylcholine and the employment of carotid or cervical sympathectomy have not been demonstrated to control the advance of the atrophic process. Along these same lines, Hamburg suggested that optic atrophy was due to lowered cellular metabolism and oxygen utilization in the optic nerves of tabetics and proposed treatment by thyroxin and by potassium permanganate intramuscularly. Loeffler used this method also with questionable results. Saitzew recommended the administration of placental blood subcutaneously in the treatment of optic atrophy because of the richness of this blood in hormones and in biologic activity.

An explanation of the pathogenesis of syphilitic optic atrophy which received considerable acceptance and support for a time at least was that advanced by Lauber and Sobanski. They contended that the atrophy results from a disturbance in the nutrition of the optic nerve or retina due to a change in the relationship between systemic blood pressure, retinal arterial blood pressure, and intra-ocular tension similar to that observed in cases of pseudoglaucoma. The normal differential between intra-ocular tension and retinal arterial diastolic pressure should be at least 14 mm. of mercury. If this differential falls, there is disturbance in the capillary circulation with resultant poor nutrition of the retina and optic nerve. All tabetics with optic atrophy suffer from peripheral hypotension, diastolic, at least, if not systolic as well. As a result, retinal diastolic arterial pressure, which averages about 45% of systemic diastolic pressure, approaches the level of intra-ocular tension, 18 to 26 mm. of mercury, circulatory failure in the optic nerve results, and optic atrophy develops. In tabetics without optic atrophy the systemic blood pressure is normal or elevated. Peripheral hypotension is caused in many instances by the presence of syphilitic aortitis with a dilated aorta. These authors assert also that all the drugs used in the treatment of syphilis, such as arsenic, bismuth, mercury, iodides, and fever therapy, tend to lower the blood pressure. Since it is impossible to maintain an elevation of the blood pressure by therapy of any sort, the treatment of syphilitic optic atrophy should be esentially that of glaucoma, miotics and, if these are ineffectual, cyclodialysis. According to Sobanski's figures, in 33 patients with optic atrophy, the differential between retinal arterial diastolic pressure and intra-ocular tension was 18 or less in 29, and less than 14 in 7. In 9 tabetics without optic atrophy, the differential was 19 or more. In his latest report on 110 eyes, 13 were blind before treatment was started and showed no improvement. Of the remaining 97 eyes, 51 improved, 26 became worse, and 20 showed no change. No patient was observed for longer than 3 years, and all had some form of anti-syphilitic treatment; some received malaria.

According to Moore and Woods, a number of observers have studied cases along the lines suggested by Lauber and Sobanski. While a few of these have confirmed their results, most have been unable to do so. There is no actual proof of the efficacy of treatment along these lines. The fact that optic atrophy does not occur in conditions such as Addison's disease with prolonged hypotension is also a strong argument against this theory. The figures presented by Langhammerova and by Ascher furnish the most convincing proof of the incorrectness of this theory. Langhammerova showed that of 59 tabetics with optic atrophy, 69.4% had normal blood pressure, 23.7% had hypertension, and only 6.7% had hypotension. Of 94 tabetics without optic atrophy, 53.2% had normal blood pressure, 22.3% had hypertension, and 24.4% had hypotension. According to Ascher, in 23 patients with tabetic optic atrophy, the systemic blood pressure averaged 133/81 in mm. of mercury, the retinal arterial diastolic pressure averaged 42.1 mm. of mercury, and the intra-ocular tension averaged 18.6 mm. of mercury. The differential then averaged 23.5 mm. of mercury. In 28 tabetics without optic atrophy, the systemic blood pressure averaged 131/82 in mm. of mercury, the retinal arterial diastolic pressure averaged 40.2 mm.

of mercury, and the intra-ocular tension averaged 19.07 mm. of mercury. The differential then averaged 21.13 mm. of mercury. These figures certainly do not lend any support to the contentions of Lauber and Sobanski.

Since the original report by Balado and Satanowsky in 1939, considerable attention has been paid to the question of the production of progressive optic atrophy by so-called optochiasmal arachnoiditis. It is assumed that, in the course of a primary inflammation of the meninges, an arachnoiditis develops around the optic nerves and chiasm. The inflammatory tissue in the arachnoid surrounds and compresses the optic nerves and chiasm and fixes them to the neighboring structures, sometimes with the collection of serous fluid in the form of arachnoidal pockets or cysts. These lesions cause constriction of the nerves and their blood supply, as a result of which atrophy develops. The only effective treatment for this condition, at least in its more advanced phases, is surgical. A certain number of cases of syphilitic optic atrophy have been included among the reports of this condition. That all cases of optic atrophy in syphilis are manifestations of optochiasmal arachnoiditis seems rather difficult to believe.

In their critical review of the reports on optochiasmal arachnoiditis in syphilitic optic atrophy, Moore and Woods state that practically all cases have been inadequately studied and that too many cases have been reported on presumption and without verification. The results of surgical treatment are not very satisfactory. However, in the light of our present knowledge, it would seem worth while to advise surgical intervention in patients with syphilitic optic atrophy, with or without classic chiasmal type field defects, if the atrophy is progressing in spite of modern antisyphilitic treatment by the subdural method or with malaria. Surgery is perhaps especially indicated in cases of optic atrophy in neurosyphilis without frank signs of tabes.

A fourth pathogenetic theory that deserves consideration is that tabes and optic atrophy are the result of combined syphilis and nutritional deficiency. Moore and Woods review the reports in the literature on the occurrence of optic nerve lesions in clinical and experimental vitamin deficiency. They conclude that it seems reasonably clear that in experimental animals at least, degenerations may occur in the spinal cord and sometimes in the optic nerves as a result of diets deficient in vitamin A, vitamin B, or both. With diets deficient in vitamin A, the degeneration is spotty in nature and is not sharply localized to the posterior columns of the cord. With diets deficient in some part of the B complex, the degeneration in the cord is sharply limited to the posterior columns and the lesion is indistinguishable from that of tabes. There is conflicting evidence as to the involvement of the optic nerves in deficiencies of vitamins A and B, but apparently degeneration of the optic nerves can be produced by some form of deficient diet. The particular component responsible is not clear as yet. The development of tabes and optic atrophy may depend conceivably on a combination of neurosyphilis and dietary deficiency, but, if so, the dietary deficiency must relate to a time period before the patient comes under observation. The deficiency of any particular element may depend on improper metabolism rather than on the absence of this element from the diet. These facts make it difficult to investigate cases of optic atrophy

clinically along the lines of nutritional deficiency. Much further study will be necessary before this theory can be accepted.

One fact that suggests that the lesion which leads to progressive atrophy of the optic nerves may be primarily inflammatory rather than primarily toxic or degenerative is that it is possible to arrest the progress of the atrophy in a certain percentage of the cases, particularly in the early stages, by modern methods of antisyphilitic treatment. In 1932, Moore stated that arsphenamine and its immediate derivatives have no deleterious effect on the structures of the eye, but that their routine use does not check the progress of the atrophy and occasionally a Jarisch-Herxheimer reaction occurs in the nerve with rapid loss of vision. Much better results are obtained, however, when arsphenamine is used by approved subdural methods. Of 138 cases of optic atrophy treated by various men by the subdural method, 54% were improved or arrested, while 46% continued to progress. Of 89 cases treated with malaria, 39% were improved or arrested, while 61% continued to progress.

With reference to the use of tryparsamide, Moore stated that 10% of the patients treated with this drug develop blurring or dimness of vision which may clear up or may remain permanent. In a few cases, after discontinuing the drug, a slowly progressive constriction of the visual fields and an increasing pallor of the optic disks may develop, which may be confused with true syphilitic optic atrophy. Differential diagnostic points are that, in the progressive atrophy developing from tryparsamide, the optic nerves and vision were normal before treatment was started, that subjectively the onset of loss of vision occurred in direct relationship to treatment, that the progress is slower than in true syphilitic atrophy, and that central vision returns to its previous level within a few weeks after the onset of the reaction and remains normal in spite of progressive constriction of the fields. Moore believes that tryparsamide is more likely to cause damage to vision in patients in whom the optic nerves are actually or potentially diseased. He treated 12 patients with optic atrophy with tryparsamide. The drug seemed to have a deleterious effect in only 4 of the 12, but it did not arrest the atrophy in the other 8. Hence, Moore is opposed to its use in cases of optic atrophy.

In a more recent paper which appeared in 1938, Moore and his coworkers present figures on the treatment of optic atrophy in which the results, though estimated on a somewhat different basis, seem even more favorable than those given in his earlier paper. The results of treatment in the later paper are estimated on the basis of the number of patients in whom the atrophy progresses to blindness within a certain period of years, "blindness" being considered as vision of 10/200, or less, in the better eye. No specific mention is made of the involvement of the peripheral fields of vision in these cases. Moore and his coworkers state that among patients with syphilitic optic atrophy who receive no treatment at all or inadequate routine treatment (which is defined as less than 10 injections of a trivalent arsenical and 10 injections of a heavy metal), 32% are blind within 1 year, more than 50% in 2 years, more than 75% in 4 years, and all except the rare patient with unusually slow progression are blind within 7 years. To be regarded as successful, any form of treatment must, in their opinion,

delay the development of blindness in a significant proportion of cases or must prevent it beyond the seventh year. When the optic atrophy is unilateral when the patient is seen first, treatment must prevent the development of blindness in the other eye. Among patients who received adequate routine treatment (defined as more than 10 injections each of a trivalent arsenical and a heavy metal), 69% were blind by the ninth year. Among patients who received subdural or intracisternal injections of arsphenaminized serum plus adequate routine treatment, only 53% were blind by the tenth year. Among patients who received malaria plus adequate routine treatment, only 14.6% were blind within 9 years. Seven of 8 patients with unilateral optic atrophy treated subdurally or with malaria did not develop atrophy in the other eye during periods varying from $2\frac{1}{2}$ to 8 years. Moore and his coworkers conclude, therefore, that the progress of primary syphilitic optic atrophy may be completely arrested by the use of either subdural treatment or malaria, more often by the latter.

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* A complete bibliography may be found in the articles by Moore and his coworkers.

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